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Contract: DA19-129-qm-1784
FMC Corporation

A Study for Resistance of Containers to Chemical Warfare Agents

Period: 24 May 1961 - 23 May 1962

QUARTERMASTER FOOD AND CONTAINER INSTITUTE FOR THE ARMED FORCES
Research and Engineering Command
Quartermaster Corps, U.S. Army
Chicago, Illinois

CONTRACT RESEARCH PROJECT REPORT

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Title of Contract: A Study for Resistance of Containers to Chemical Warfare Agents

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Organic Chemicals Division
FMC Corporation
Baltimore, Maryland

Final Report - May, 1962

I. INTRODUCTION

The scope of Contract DA19-129-QM-1784 (01 5091), "A Study for Resistance of Containers to Chemical Warfare Agents" was defined in the contract as follows:

> "The Contractor shall, commencing on 24 May 1961 and continuing through 23 May 1962, supply the necessary personnel, facilities and materials and do all other things necessary for or incident to and will furnish his best efforts within the estimated cost to the performance of the work as set forth below:
>
> "(1) The Contractor shall determine adequacy of current laboratory techniques and procedures for testing materials for resistance to penetration of chemical warfare agents.
>
> "(2) Where necessary, the Contractor shall modify current laboratory testing methods or develop new methods to assure precise measurement of the degree of effectiveness of the materials in preventing penetration of the agents.
>
> "(3) The Contractor shall determine the resistance of approximately twenty packaging materials using techniques described in paragraphs (1) and (2), above. Materials will include but not necessarily be limited to unsupported plastic films, aluminum foil, paper and combinations thereof. The Project Officer will determine the specific materials to be evaluated, which materials shall be furnished by the Government to the Contractor at Government expense.
>
> "(4) The number of chemical warfare agents to be tested will not exceed five (5). The specific warfare agents will be determined by Army Chemical Center, Edgewood, Maryland, in consultation with the Contractor and the Project Officer. The agents shall be furnished by the Government to the Contractor at Government expense."

The Project Officer for this contract was Mr. Frank J. Rubinate (Chief, Development and Standards Branch, Container Division, Quartermaster Food and Container Institute), who made the choice of packaging materials to be tested, and exercised general supervision of the project.

The agents to be tested were specified by the U. S. Army Chemical Research and Development Laboratories. Miss Virginia Bauer of the Biochemical Research Division was designated as technical advisor representing the Chemical Research and Development Laboratories.

II. SUMMARY

An apparatus was designed to allow a direct comparison of the permeabilities of the packaging materials towards chemical warfare agents. A sensitive analytical method for each of the five chemical agents was adapted to the test requirements. Vapor permeation tests were capable of distinguishing barrier efficiencies only for the more volatile agents. A standard liquid permeation test was therefore adopted as a means of barrier efficiency evaluation, applicable to all packaging materials and agents.

Test conditions comprised placing 0.04 g. of each agent directly upon the circular film test piece, forming a barrier between two compartments. The agent permeated was collected in a solution in the lower compartment. Samples of the collecting solution were analyzed over a 10-14 day period. The tests were conducted at room temperature and 50 percent relative humidity.

The degree of permeation was expressed as micrograms of agent permeated per 100 sq.in. of barrier during the test period. Values so obtained allow a direct comparison of barrier efficiencies under test

conditions. Substantial differences in permeability were observed. The test is regarded as a practical screening method for choosing packaging materials for more rigorous testing under variable field conditions.

The relative permeabilities of the packaging materials are summarized in Table I, p. 4. Qualitative observations on each type of material in the approximate order of efficiency are listed below:

A. Vinyl-Aluminum Foil-Mylar Laminate: Excellent resistance to all agents.

B. Mylar: Generally impermeable except toward Agent I.

C. V3 Board (paper laminate including two asphalt layers): Intermediate permeability.

D. Saran: Certain samples showed good inherent resistance but wide variations in results indicate a lack of uniformity.

E. Cellophane coated with Saran: Rather poor resistance to permeation with the possible exception of Agent I.

F. Polyethylene: Rather poor resistance.

G. Cellophane: Poor resistance.

H. Kraft Board: Poor resistance.

I. Sulphite Board: Poor resistance.

In Table I, and throughout the report, the agents are designated by number, I through V. The corresponding agent identification is listed in Table III, p. 6. In Table II, p. 5, the packaging materials are identified.

Table I

Comparative Permeabilities of Selected Packaging Materials (a)

Film No.	Material	AGENT I	II	III	IV	V
1	Sulphite Bd.	5	6	7	6	6
2	Mylar	2	1	1		2
3	Foil Laminate	1	1	1		2
4	Kraft Bd.	5	6	6	6	6
5	V3 Bd.	3	2	3		2
6	Saran, Type 7	2	3	2	5	5
7	Saran, Type 17	1	3	1		6
8	Saran-coated Cellophane -DK-202	1	4	4		5
9	Saran, Q4164.7	5	4	5	4	4
10	Cellophane,195 PSD 12412	5	6	6	5	6
11	Foil Laminate (Dobeck)	1	1,1	3		
12	Foil Laminate (Dobeck)	1	1			
13	Foil Laminate (MilPrint)	1	3			
14	Foil Laminate (Con-Can)	1	6,1	1		
15	Foil Laminate (Kleerpak)	1	1			
16	Foil Laminate (MMM)	1	1,2	1		
17	Mylar (DuPont)	3	1,1	3		
18	Cello-Saran (Am.Viscose)	5	6,5	5		
19	Cello-Saran (DuPont)	2	4,3	4		
20	P.E. Low (DuPont)		5	5		
21	P.E. Med (DuPont)		4	5		
22	P.E. Low (Carbide)		5	5		
23	P.E. Med (Carbide)		4	5		

(a) Table I was prepared by averaging the results shown in Tables XI through XV, pages 58 through 62 . The rating numbers listed in the agent columns have physical significance, i.e., a rating of 1 indicates less than 10 micrograms permeated per 100 sq.in.; a rating of 6 indicates less than 10^6 micrograms but more than 10^5 micrograms permeated.

Table II

Selected Test Packaging Materials

Material No.	Supplier	Type of Film	Notes
1	-	Sulphite Board	22 pt.,white, porous cardboard
2	DuPont	Mylar Film	1 mil
3	DuPont	Foil Laminate	0.003 vinyl-0.00035 alum.foil 0.0005 Mylar
4	-	Kraft Board	Brown, porous cardboard
5	-	V3 Board	40 pt.,multi-layer, two asphalt layers
6		Saran Film	Type 7, 100 gauge
7		Saran Film	Type 17, 100 gauge
8	DuPont	Saran-coated Cellophane Film	DK-202 - 1/2 mil Saran coating
9		Saran Film	Q4164.7, 100 gauge
10	DuPont	Cellophane Film	195, PSD 12412
11	Dobeckmun Div., Dow	Foil Laminate(a)	(VBA 1142) Vinyl-Alum.Foil-Mylar, Lot 1
12	" "	" " (a)	" " " " Lot 2
13	MilPrint	" " (a)	(F-11)" " " " Lot 1
14	Con.Can	" " (a)	Vinyl " " " Lot 1
15	Kleerpak	" " (a)	" " " " Lot 1
16	Minn.Min. & Mfg.	" " (a)	" " " " Lot 1
17	DuPont	Mylar Film	Lot No. 1, 1.0 mil
18	Am.Viscose	Saran-coated Cellophane	Lot No. 1, RS-2, 140 gauge
19	DuPont	Saran-coated Cellophane Film	Lot No. 2, K-202, 140 gauge
20	DuPont	Polyethylene Film	Low Density, 200 A-101
21	DuPont	Polyethylene Film	Medium Density
22	Union Carbide	Polyethylene Film	Low Density, N-695-M
23	Union Carbide	Polyethylene Film	Medium Density, N-690-A

(a) These laminates were all composed of 0.5 mil Mylar, 0.35 mil foil and 3.0 mil vinyl.

Classified page #6 has been removed from this report and is not available for distribution.

III. RECOMMENDATIONS FOR FUTURE WORK

A. More Complete Tests of the More Resistant Materials

The tests described in this report were made by determining the permeation of measured quantities of liquid agent deposited on the surface of the material being examined without regard to the area of liquid contact. This procedure led to a satisfactory differentiation between films with appreciable differences in permeability, and permitted conclusions to be drawn as to the preferential use of a specific packaging material.

It would also be desirable to carry out similar tests in which the area exposed to liquid agent would be held constant. This test variation would provide more direct information concerning the rate at which an agent penetrated any specific packaging material. This value would then make possible a more reliable estimation of the length of time during which a specific packaging material would offer protection against penetration by agents.

B. Tests of Packaging Materials After Their Having Been Subjected to the Packaging Processes

Each of the packaging techniques below would be expected to offer a possibility of adversely affecting the barrier efficiency of test materials. Tests of the effects of these factors upon the better films would be recommended.

1. Creasing.
2. Folding.
3. Cornering.
4. Heat sealing, where applicable.
5. Glueing, where applicable.

6. Multi-layer assemblages of packaging materials in the order used for packaging.

7. Handling of completed packages, as represented by tumbling and falling tests.

C. Effects of Climatic Variations

Tests to date have been conducted at room temperature (70-75°F.) and 50 percent relative humidity. Variations in temperature and humidity may greatly affect permeability directly (B-1) and indirectly in terms of physical strength and resilience of the packaging material. In any extension of this work, it is recommended that the more promising of the packaging materials be tested under conditions representing the complete range of climatic conditions reasonably to be expected.

IV. DISCUSSION OF RESULTS

A. General Results of the Permeability Tests

The twenty-three samples of various packaging materials shown in Table II, page 5. were tested for resistance to permeation by the selected chemical agents shown in Table III, page 6. The first ten materials were tested initially for resistance to each of five chemical agents. Additional samples of those materials found to be least permeable were then tested for resistance to permeation by two of the five chemical agents. These additional tests thus provided an indication of the reproducibility to be expected from different suppliers, or production lots, of each type of film. The condensed results of these tests are shown in Table I, page 4. Most of the tests were made in duplicate in order to provide additional information

concerning the reliability of the analytical procedures and the uniformity of the test materials.

As described in Section VI, page 41, Experimental Procedures, the tests were carried out in an apparatus consisting of an open bell jar-shaped chamber and a dish-shaped collecting chamber which could be clamped together by means of opposing ground glass flanges. The film to be tested was inserted as a barrier between these two sections. The chemical agent to be used was placed in the upright cylindrical chamber; any agent which passed through the film was collected in the second chamber and measured analytically. Tests were made of the permeability of the first ten materials toward chemical agents in the vapor phase as well as in the liquid phase. These tests, in general, confirmed the results of the liquid tests, but were not applicable to the less volatile agents. During the latter part of the program, the experiments were therefore limited to tests with agents in the liquid phase. The same test cell was used for both the liquid phase and the vapor phase tests, except that, for the vapor tests, agent vapor was allowed to generate from a supply of agent suspended in the chamber above the test film barrier as shown in Figure 7, page 43. In the liquid permeation tests, the amount of agent placed on the film (0.04 ml.) was held constant throughout the entire series. The test cells were adjusted so that the film surfaces were horizontal and, in general, the agent remained spread out in the center of the film. No attempt was made to measure the area covered or to make the area covered uniform.

It was felt that the tendency of agent drops to spread upon contact with the film surface would be a characteristic of the test film itself and valuable results would be obtained by keeping constant the amount of agent added.

The penetration of the permeable films by agent vapor was characterized by an induction period during which negligible quantities were detected in the collecting solution. The concentration in the collecting chamber then rose rapidly to a maximum at which it remained relatively constant. The lack of any further increase in concentration in the collecting chamber was undoubtedly associated with a simultaneous decomposition of the agent in the vapor chamber. Gradual decomposition of agents under humid conditions was to be expected. The results of vapor phase tests with the sulphite board, kraft board and with cellophane are shown in Figures 8, 9 and 10, respectively.

The permeation data was expressed in terms of micrograms of agent permeated per 100 square inches of barrier. Because of the limitation of the analytical methods, values below a specific minimum level which varied with each agent were considered to be without significance. All such values obtained were nevertheless recorded in the tables even for the barriers resisting penetration. The table entries considered to be below the sensitivity of the method were enclosed in parentheses.

As shown in Tables XI through XV, pages 58 through 62, the tests have given fairly consistent results. The laminated aluminum foil, Mylar and Saran Wrap (Type 17), respectively,

appeared to be most resistant to permeation. The V3 board was also relatively resistant to permeation by the agents in general. The sulphite and Kraft boards, Saran Q4164 and Cellophane offered little or no resistance to permeation. The Saran Type 7 and Saran-coated cellophane appeared to give erratic results which were attributed to a lack of uniformity in the film samples. Both low density and medium density polyethylene were found to be quite permeable toward the only two of the agents against which they were tested.

B. Relative Permeabilities of the Types of Materials Tested

1. White Sulphite Board
This porous cardboard was readily permeable toward all five of the agents tested, as shown in Figure 14, page 93. The material was intended for use, however, as an internal packaging wall and was not designed to provide resistance to external permeation.

2. Mylar
This polyester film was one of the better materials tested. It was apparently permeated to some extent by Agent I and permitted up to 100 micrograms per 100 square inches to penetrate during a 10-15-day test period.

3. Vinyl-Aluminum Foil-Mylar Laminate
This material, an aluminum foil laminated internally with a vinyl film and, externally, with Mylar, was the most resistant film tested. A total of seven samples of this product, representing different suppliers or production lots, were examined. Only one sample, No. 14, was permeable; a second sample from this supplier was impermeable.

4. Kraft Board
This product was similar in appearance and composition to No. 1, the sulphite board, and was found, as shown in Figure 14, page 93, also to offer little resistance to permeation by any of the agents tested.

5. V3 Board
This material was a heavy, multi-layered cardboard in which were included two layers of asphalt. It was apparently designed to provide packaging strength and some degree of impermeability toward moisture, etc. The product provided a surprising resistance to permeation by the five agents tested.

6. Saran, Type 7, was found to be only intermediate in resistance to permeation by the five agents.

Saran, Type 17 was much less permeable than Type 7, but an appreciable variation in the test results was observed. This may have been attributable to a lack of uniformity in the film tested.

Saran, Q4164.7 was much more permeable than Type 7 and offered comparatively little resistance to permeation by any of the five agents, as shown in Figure 13, page 92.

7. Saran-coated cellophane
Three different lots of Saran-coated cellophane were found, generally, to offer little resistance to permeation except that permeation by Agent I was found to be relatively low for two of the three samples.

6. Cellophane
The single sample of cellophane tested offered almost no resistance to permeation by any of the five agents, as indicated in Figure 12, page 91.

9. Polyethylene
Neither low density nor medium density polyethylene films offered any appreciable resistance to permeation by the two agents with which tests were made, Nos. II and III. These results are shown in Figure No. 11, page 90.

C. Limitations of the Test Procedures
The small quantities of agents which were found to penetrate the less permeable films studied during these tests imposed certain limitations on the results obtained. Since less than 1-10 micrograms of agent were found to penetrate the better films during the entire test period, intermittent analyses of these particular systems was only possible with extremely sensitive analytical methods. Since three of the five agents used were cholinesterase inhibitors, it was possible to use a standard enzymatic inhibition procedure for the detection of these three agents. However, this analytical procedure was difficult to control and was characterized by the limited reproducibility associated with many extremely sensitive methods.

It was necessary to use colorimetric procedures also for both agents IV and V. The procedure for Agent IV was found to be insufficiently sensitive to differentiate between the less permeable films tested. It was necessary to regard these results as simply indicating that less than a given minimum quantity of

agent permeated the film during the test period.

Agent V was found to decompose upon standing in the collecting solution used and it was necessary to use an analytical procedure which would detect the decomposition products.

These analytical procedures were all sufficiently accurate, however, to distinguish between those films which were relatively impermeable and those which offered little resistance to permeation by the agents. Those values which were so low as to be considered without significance were shown in parentheses in the accompanying tables of results.

In addition to the limitations inherent in the analytical methods, other variations were imposed by the properties of the agents and the mechanical procedures followed. Humidity is known to be an important factor affecting permeability (B-1). The relative humidity of the test chamber was arbitrarily controlled at 50 percent, by means of an aqueous solution of propylene glycol used as collecting solution. The agents in general are subject to hydrolysis; hence, over the period of the test a slow but significant hydrolysis probably occurred, tending to give somewhat low results for permeability. Stability tests of each agent in the collecting solution were made and are discussed in later sections.

In addition to hydrolytic losses, certain agents are known to be subject to deterioration in storage. Agent I, for instance, is subject to rapid deterioration in a thin film in contant with glass. This behavior was exhibited in the vapor permeability tests

with Agent I (p.87-9), wherein it is apparent that a constant saturated vapor concentration was not maintained in the vapor generator side of the barrier throughout the test period. In most of the tests, the liquid agent was placed directly on the barrier film. Although it is probable that plastic surfaces are much less disruptive than glass, individual stability tests have not been made.

Since vapor permeability tests were incapable of differentiating between the less permeable films, it was necessary to place the liquid agents directly on the film. Although the magnitude of the deposits were maintained approximately constant, variable wetting of the film, tilting, etc. undoubtedly caused variations in the area exposed, as mentioned earlier.

Finally, the number of specimens tested are probably inadequate statistically to eliminate, unequivocally, the effects of occasional analytical errors and film imperfections introduced during manufacturing.

While it is recognized that these limitations may affect the accuracy of the absolute amount of agent permeated, the results possess integrity and are entirely adequate for the purpose of screening film types and choosing suitable candidates for more rigorous field testing.

V. DEVELOPMENT OF ANALYTICAL PROCEDURES

A. Adaptability of the Enzymatic Procedure to the Study of the Permeability of Films

A number of advantages led to the selection of the enzymatic procedure for use in the proposed study of permeability of films

by those agents possessing the property of cholinesterase inhibition. The procedure was extremely sensitive and, after some modifications were made, proved to be capable of detecting as little as 0.001 micrograms/ml. of agent. Since the quantity of agent penetrating several of the better packaging materials could be expected to be very low, such sensitivity was highly desirable.

Also, the procedure was only responsive to materials which still retained toxicity after penetration of the film. Since contamination of the packaged materials by toxic substances was the really important question being studied, this appeared to be a distinct advantage. The enzymatic process also appeared to be usable for the analysis of test systems involving three of the five agents to be studied initially. This minimized the work involved in analytical procedural development and standardization.

The selection of the enzymatic method of analysis required consideration of several factors in order to modify it for use in the proposed program. As indicated above, the procedure involved first establishing a controlled reaction system and adding test solutions to this system until a measurable effect on the enzymatic activity is observed. This involved the preparation and testing of an entire, continuous series of dilutions, of the unknown solution, to be certain of obtaining at least one dilution which fell within

the range of the test conditions. The procedure, as it was previously carried out in these laboratories, only permitted the analysis of 15-20 samples per day and, until some knowledge of the strength of the unknown solution was obtained, it was quite possible to carry out an entire day's analyses and still not have a reliable value for the unknown concentration. As indicated below, this problem was minimized by a modification of the procedure which permitted the analysis of a larger number of samples.

The enzymatic procedure was also subject to interference by extraneous solvents, etc. This problem appeared to be satisfacotrily controlled. Although propylene glycol did interfere, if present in high concentrations, dilution of the propylene glycol to less than about 10 percent in the test solution was successful in preliminary test work. This interference, and its successful elimination, are shown in Table IV. Solvent concentrations were chosen such that inhibition due to solvent was less than 10 percent.

1. Procedural Changes

When the enzymatic method for the determination of Agent No. 1 was initially proposed for use in the film permeability program, it became apparent that the procedure should be modified so as to permit the simultaneous analysis of a much larger number of samples. A review of the proposed work under the Quartermaster Corps Contract indicated that it would be desirable to be able to analyze as many as 100-150 solutions per day.

Several procedural modifications were proposed and found to be satisfactory. These included:

a. Substitution of unstoppered 15 x 150 mm. test tubes for the stoppered 10 ml. volumetric flasks previously used. This small change permitted the use of a suspended test tube basket rather than the individually suspended volumetric flasks, and also simplified the time-consuming job of washing glassware.

b. Elimination of the process of addition of reagents at exact one-minute intervals. The reagents were added in rapid succession to the entire series of samples involved.

c. Use of stronger trichloroacetic acid for neutralization of the reaction.

d. Minor procedural changes designed to speed up the processing of increased numbers of samples.

These modifications were satisfactory as it was found that 40-50 samples could be analyzed within a two to three hour period.

The detailed enzymatic procedure used is recorded in Section VI-E, p. 50.

Table IV

Percent Inhibition of Acetylcholinesterase Due To Propylene Glycol Under the Enzymatic Test Conditions

Sample No.	Source	Dilution	Aliquot (a)	Percent Inhibition
1	78.5% P. Glycol	x 10	0.5	0
2	"	"	1.0	0
3	"	"	1.0	0
4	"	"	1.0	2.8
5	"	"	1.5	12.5
6	"	"	2.0	10.9
7	"	"	2.0	5.6
8	"	"	2.0	5.6
9	"	"	2.5	10.9
10	"	"	3.0	11.0
11	"	"	3.0	11.0
12	"	"	3.0	15.5

(a) Water was added to these aliquots, where necessary, to make a total volume of 3.0 ml. which was then analyzed using the enzymatic procedure. Thus, the values shown in column 5 represent the inhibitions which were found to be due to propylene glycol in the absence of any agent.

B. Use of the Enzymatic Procedure with Agent No. I

1. Stability of Agent No. I in Aqueous Propylene Glycol

In order to use the enzymatic procedure for the determination of Agent No. I in the collecting solution, it was necessary that the agent remain stable in the collecting solution through the period of the test. The stability in aqueous, acidic, 78 percent propylene glycol was established by preparing a series of solutions of known concentration and analyzing these solutions intermittently.

The stability of Agent I in these solutions was found to be satisfactory as shown by the series of successive analyses listed in Tables V and VI, pages 22 and 23. Some degree of interference may have been observed in the series of analyses, particularly in the more dilute solution. A consistent analysis of about 0.001 microgram/ml. was obtained for this solution. It was uncertain whether or not this high result was due to an error in preparation, interference by the propylene glycol or to some other factor. The propylene glycol interference, when observed, was found to be erratic as shown by the three 10th-day analyses in Table V, page 22. The use of dilute sulfuric acid was selected in order to eliminate any partial pressure of agent from the collection solution itself.

2. Preparation of a Calibration Curve for Agent No. I

Since the stability of Agent No. I in aqueous 78 percent propylene glycol was apparently satisfactory, a series of solutions of known concentration in aqueous, acidic propylene

glycol were prepared and analyzed. The dilution and analytical procedures were carried out, as nearly as possible, in the manner in which the analyses of the test cell samples were made.

These results were plotted so as to give the calibration curve shown in Figure 1.

This curve was checked again later in the program using a supply of stabilized Agent I. The slope of the line was decreased somewhat because of dilution by the stabilizer, but the stabilizer did not interfere with the enzymatic analytical procedure.

C. Use of the Enzymatic Procedure For Agent II.

1. Stability of Agent II in Aqueous Solution

It was necessary, again, that the agent remain stable in dilute aqueous solutions for at least 1-2 weeks in order to use the enzymatic analytical procedure since the method only detects the presence of active agent.

The decomposition of Agent II in aqueous solution has been described as perhaps taking place more readily than that of Agent I and as being catalyzed by both acids and bases. It was suggested (Miss V. E. Bauer, Army Chemical Center, Chemical Research and Development Laboratory) that improved stability might be effected, if necessary, by the use of a buffered collecting solution (pH 4-6).

In order to provide a preliminary indication of the stability of this agent, solutions of Agent II in water,

Table V

Stability of Agent I in 78.5 Percent Propylene Glycol

Reference	No. Days	0.0005 (a)	0.0010 (a)	0.0015 (a)	0.0020 (a)
D495-79	0	0.0010	0.0020	0.0030	0.0027
D495-82	1	0.0010	0.0015	0.0017	0.0023
D495-83	2	0.0008	0.0012	0.0016	0.0022
D495-85	3	0.0010	0.0013	0.0017	0.0023
D495-93	7	0.0008	0.0010	0.0014	0.0017
D495-98	9	0.0010	0.0013	0.0017	0.0020
D495-99	10	0.0015	0.0015	0.0015	0.0022
D495-99	10	0.0013	0.0011	0.0019	0.0017
D495-99	10	0.0011	0.0012	0.0018	0.0018

(a) Actual concentration of test solution, in micrograms/ml. The values tabulated in each column are the concentrations found by analysis at the indicated time intervals.

Table VI

Stability of Agent I in Dilute Aqueous H_2SO_4 (a)

Reference	No. Days	0.0005(b)	.0010(b)	.0015(b)	.0020(b)
D495-82	5	0.0006	.0011	.0014	.0017
D495-83	6	0.0005	.0010	.0014	.0017
D495-85	7	0.0006	.0011	.0014	.0017
D495-89	8	--	.0010 (c)	--	--
D495-93	11	0.0005	.0010	.0014	.0016
D495-94	12	0.0005	.0010	.0015	.0018

(a) Prepared by removing appropriate aliquots from an Agent I solution in 0.001 N H_2SO_4 (0.1 mg/ml.) and diluting with water.

(b) Actual concentrations of test solutions, in micrograms per ml. The values tabulated in each column are the concentrations found by analysis at the indicated time intervals.

(c) The average result of 20 replicate analyses.

in aqueous propylene glycol and in buffered (pH 5.6) aqueous propylene glycol were prepared and analyzed after standing for a period of four days. The solution in aqueous propylene glycol was found to be sufficiently stable, on a preliminary basis, to justify making a more detailed investigation of its use in this program. These preliminary results are shown in Table VII, page 25, in terms of percent inhibition as a function of concentration before and after aging.

A solution of Agent II in aqueous 78 percent propylene glycol was prepared and analyzed intermittently for a period of ten days. As shown in Table VIII, Agent II was apparently sufficiently stable in aqueous propylene glycol to permit the use of the enzymatic procedure for the analysis of the collecting solutions, even though slow deterioration is apparent.

2. Preparation of a Calibration Curve for Agent II

A calibration curve for Agent II was prepared by analyzing a solution of Agent II of the maximum concentration that might be reached in a test cell collecting solution (about 0.011 micrograms/ml.). The dilutions and analyses were carried out using exactly the same procedure followed for diluting and analyzing the test cell solutions (See Section IV, Experimental Procedures). The results are shown in Figure 2, p. 29.

Table VII

Stability of Agent II in Aqueous Solutions (a)

	Micrograms of Agent II	Solvent: H_2O % Inhibition	Solvent: 22% H_2O 78% Propylene Glycol % Inhibition	Solvent: 22% Buffer Solution (b) 78% Propylene Glycol % Inhibition
Original Analysis	0.0054	24	27	30
	0.0038	17	15	25
	0.0027	11	11	14
	0.0019	9	7	13
	0.0013	7	6	10
Percent Inhibition After 4 Days		22	21	22
		14	13	15
		7	14	13
		5	5	7
		3	2	2

(a) This was a preliminary study designed to indicate the general stability of Agent II. A more detailed study was then made comparing aqueous propylene glycol and buffered aqueous propylene glycol.

(b) Prepared by dissolving 2.9 g. of trisodium citrate and 0.64 g. of citric acid in 100 ml. of water (pH = 5.6).

Table VIII

Stability of Agent II in Aqueous 78 Percent Propylene Glycol

Micrograms of Agent II	% Inhibition After No. of Days Indicated (a)					
	1	2	4	7	8	10
.033	100.0	100.0	100.0	91.0	98.5	79.3
.023	93.8	85.0	88.8	83.4	94.8	74.5
.016	85.8	77.5	86.0	79.0	69.6	64.7
.011	79.5	60.0	76.3	67.0	65.0	54.9
.008	61.5	48.8	70.8	53.6	54.5	43.9
.006	55.5	37.2	58.3	52.2	43.9	35.4
.004	42.5	18.6	47.2	28.4	30.3	28.1
.003	--	20.0	41.6	25.4	21.2	19.5

(a) A stock solution originally containing 0.11 micrograms of Agent II per ml. of 70 per cent propylene glycol in water was diluted 1:10 with water. An aliquot of 3.0 ml. was removed for analysis. A second aliquot portion of 70 ml. was diluted to 100 ml. and a 3.0 ml. sample removed. This 70:100 dilution process was repeated until the eight samples indicated were obtained.

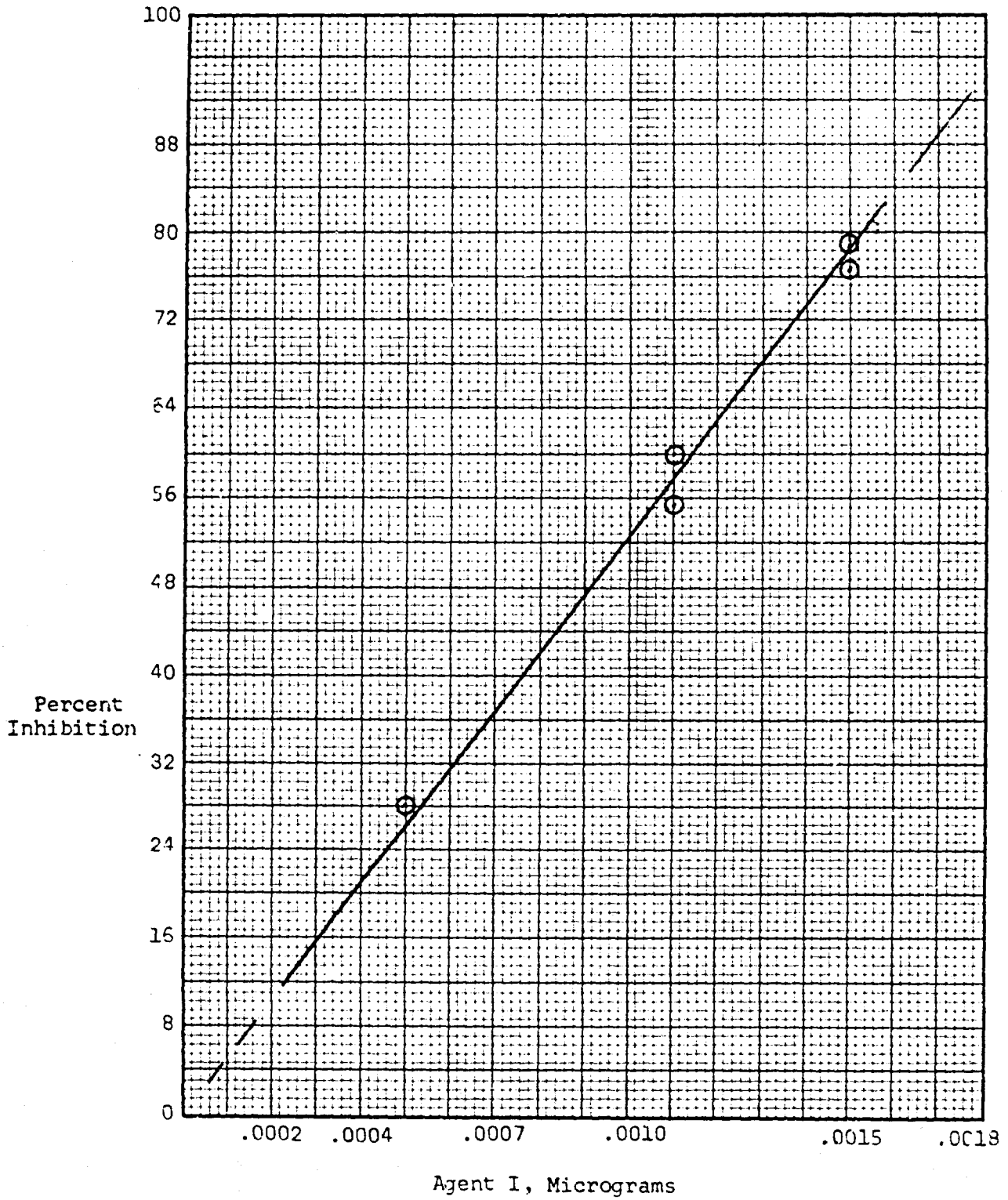


Figure I - Calibration Curve for Agent I.

D. Use of the Enzymatic Procedure for Work with Agent III

1. Stability of Agent III in Aqueous 78 Percent Propylene Glycol

The use of the enzymatic analytical method for work with Agent III was considered desirable, as previously with Agent II, because of the extreme sensitivity of the procedure. Since the procedure is only responsive to active agent, however, it was again necessary that Agent III be inherently stable for a period of one or two weeks in the aqueous propylene glycol used as a collecting solution.

In order to test this stability, a solution of Agent III in aqueous 78 percent propylene glycol was prepared and analyzed at intervals for a period of about two weeks. The results are shown in Table IX. In order to also evaluate the reproducibility of the results, several analyses were made of freshly prepared solutions of Agent III. These results are also shown in Table IX.

The stability of Agent III in aqueous propylene glycol, while not good, was considered adequate for our test purposes.

2. Preparation of a Calibration Curve for Agent III

A calibration curve for use in the studies with Agent III was prepared by analyzing a fresh solution (at zero days) as shown in Table IX. The resulting calibration curve is shown in Figure 3. While the reproducibility to be expected from the use of this curve would not be of the highest order, as shown by an inspection of Table IX, it should be entirely adequate for our purposes. Again, a slow deterioration of agent in solution is apparent.

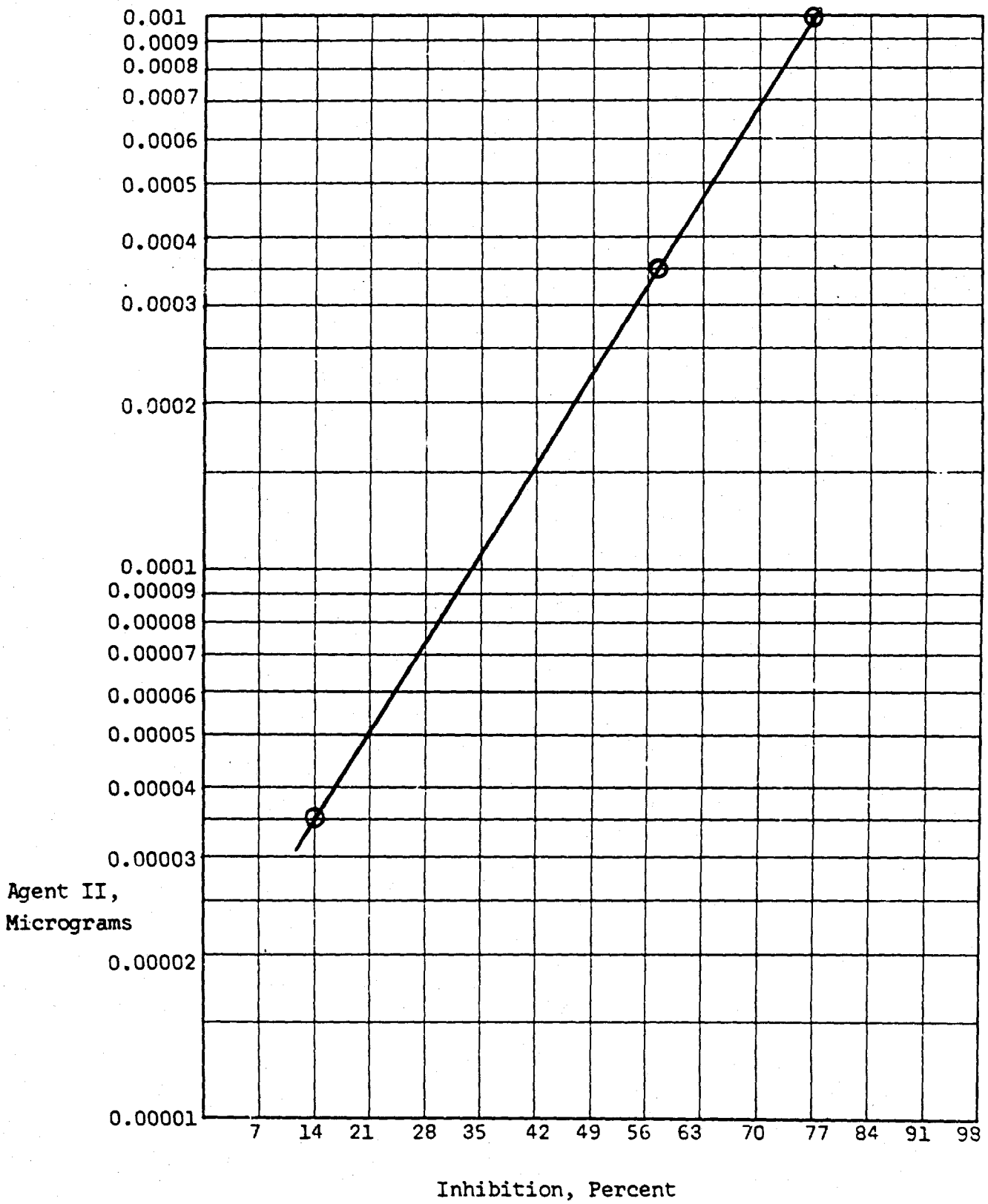


Figure 2 - Calibration Curve For Agent II.

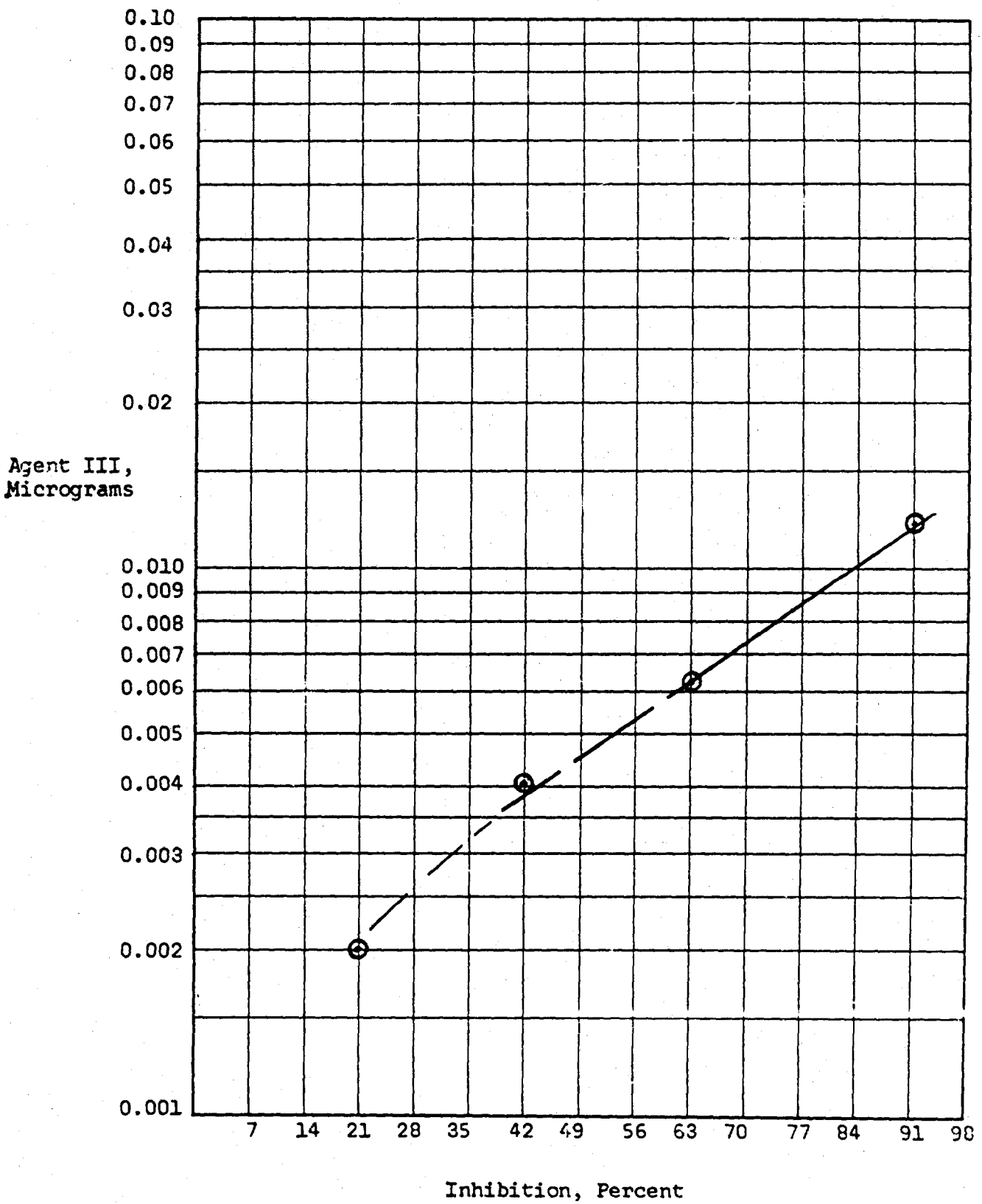


Figure 3 - Calibration Curve For Agent III.

Table IX

Stability of Agent III in Aqueous Propylene Glycol

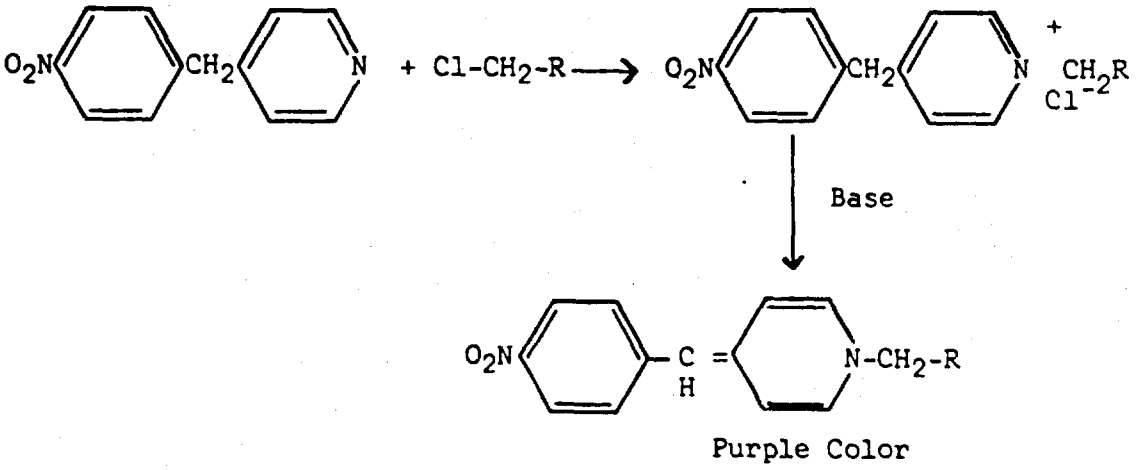
Micrograms of Agent III (a)	Percent Inhibition After Number of Day's Storage Indicated			
	0	1	9	13
0.039	>100, >100	100	93, 100	94, 75
0.0039	86, 62	98	38, 44	15, 6
0.00039	15	22	12, 15	--
0.036	94, >100, >100			
0.0036	13, 45, 44			
0.0027	11, 34, 28			
0.0018	7, 14, 19			
0.0009	7, 5, 8			
0.0005	4, 5, 6			
0.00025	4, 5, 2			

(a) These values represent the actual concentrations of the test solutions. The analytical results tabulated represent the equivalent inhibition after the time intervals indicated.

E. Development of An Analytical Procedure for Use With Agent IV

1. Analytical Procedural Development

Since Agents IV and V are both vesicants and are not effective cholinesterase inhibitors, it was necessary to adopt a different analytical procedure for analyzing the collecting solutions used in our test systems. The analytical reaction used for the detection of both Agents IV and V is well known and is based upon the alkylation of 4-(p-nitrobenzyl)pyridine (DB-3) as shown below. The quaternary pyridinium salt formed is converted, in alkaline solution, to a highly colored complex which can be measured spectrophotometrically.



Purple Color

The reaction is rather straightforward and most of the recent references (B-2, B-3) have involved the adaptation of the reaction to the particular system being studied or the specific compound being detected.

It was considered highly desirable to continue to use 78 percent propylene glycol in water as the collecting solution in our test system, since its use leads to a relative humidity of about 50 percent in the system. It was decided, therefore, to attempt to use aqueous 78 percent propylene glycol as the solvent for the analytical reagents also. This modification gave good results and was adopted. A number of alkaline reagents can be used for the color development step. Piperidine has been used widely for this purpose and has the advantage of being readily miscible with the desired 78 percent propylene glycol reaction medium. The following procedure was, therefore, adopted arbitrarily and has given entirely acceptable results.

Procedure

To 5 ml. of a 78 percent solution of propylene glycol in water, containing about 75 micrograms or less of Agent IV, was added 5 ml. of DB-3 reagent (prepared by dissolving 3.0 grams of 4-(p-nitrobenzyl)pyridine and 0.5 gm. of potassium perchlorate in 100 ml. of a 78 percent solution of propylene glycol in water).

To the solution, after heating in a boiling water bath for four minutes and cooling for two minutes in an ice bath, were added 25 drops of piperidine. After one minute, the solution was read at 540 millimicrons. A blank solution was prepared by carrying out the procedure using 5.0 ml. of aqueous 78 percent propylene glycol containing no agent, 5 ml. of DB-3 reagent and 25 drops of piperidine.

2. Stability of Agent IV in Aqueous Propylene Glycol
It was considered necessary that Agent IV remain stable in aqueous propylene glycol for a period of one or two weeks since the analytical procedure was designed to detect undecomposed agent. A solution of Agent IV in aqueous 78 percent propylene glycol was prepared and analyzed at intervals for a period of about one week. As indicated in Table X, the stability of Agent IV was considered adequate under these conditions.

3. Preparation of a Calibration Curve for Agent IV
Since the intermittent analysis of the solution of Agent IV indicated that the results would be comparatively reproducible, the results obtained from the analysis of the freshly prepared solution were used for the calibration curve shown in Figure 4.

As was expected, a comparison of the relative permeabilities of the more resistant films was not possible during these test series with Agent IV. This was due to a lack of the required sensitivity on the part of the analytical procedure. As seen in Figure 4, the minimum amount of Agent IV which can be measured with minimum reliability was about 1-2 micrograms, whereas the detection of about 0.01 to 0.1 micrograms would be necessary in order to distinguish between the better films being tested.

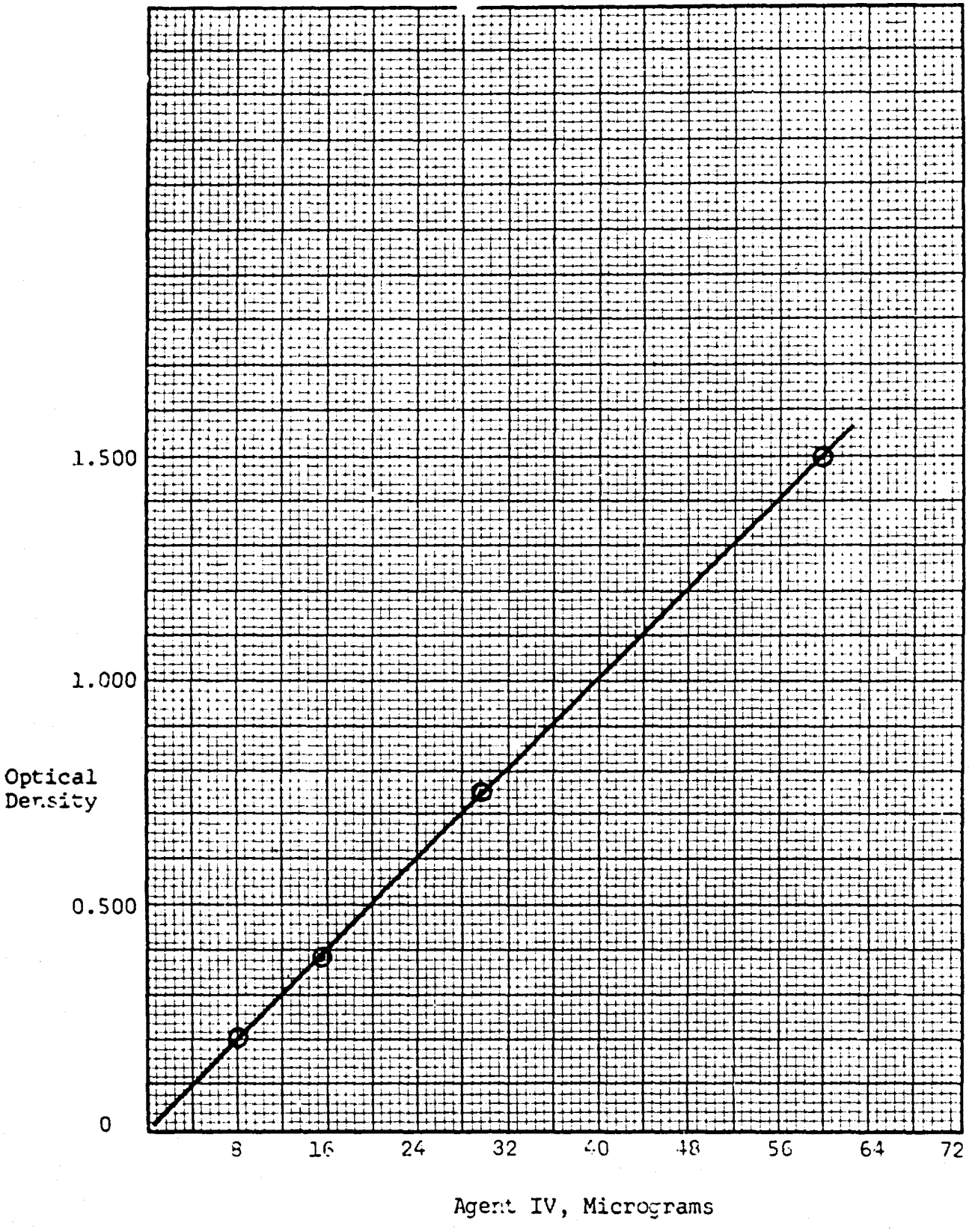


Figure 4 - Calibration Curve For Agent IV.

Table X

Stability of Agent IV in Aqueous 78 Percent Propylene Glycol (a)

No. of Days of Storage	Optical Density Equivalent to Indicated Micrograms of Agent IV			
	60	30	15	7.5
0	1.500	0.830	0.460	0.230
1	1.500	0.750	0.395	0.210
2	1.250	0.740	0.370	0.210
3	1.250	0.750	0.400	0.210
4	1.500	0.850	0.500	0.290
7	0.900	0.620	0.360	0.220
8	0.900	0.520	0.290	0.160

(a) These results were obtained using the DB-3 procedure. While a slow decomposition of the agent is evident, the stability was considered adequate for these tests.

F. Development of An Analytical Procedure for Use With Agent V

1. Stability of Agent V in Aqueous Propylene Glycol

The DB-3 procedure used for the analysis of Agent IV can also be used for Agent V. Since the DB-3 procedure was only sensitive to undecomposed agent, however, it was again necessary that Agent V remain stable in aqueous propylene glycol for the one to two-week test period.

In order to test this stability, a solution of Agent V in 78 percent propylene glycol was prepared and analyzed after standing. As shown in Figure 5, Agent V was found to have decomposed extensively in this solution after standing for only one day. The DB-3 procedure could not be used, therefore, for this agent under these conditions, and it was necessary to resort to an alternate analytical procedure which would detect Agent V after decomposition.

2. Analytical Procedure for Agent V

An alternate procedure (V. E. Bauer, Army Chemical Center) was then tested for use with Agent V. This procedure is based upon the oxidation of the sulfur in Agent V and is independent of the extent of decomposition of the agent.

Determination of Agent V and/or Decomposition Products

To 5.0 ml. of a 78 percent solution of propylene glycol in water, containing 50-60 micrograms or less of Agent V, was added 1.0 ml. of 4 N hydrochloric acid and 1.0 ml. of a 0.04 percent solution of chloramine T in water. After the resulting solution had been allowed to stand for five minutes, 1.0 ml. of 0.1 M potassium iodide in water was added. After an additional three minutes, the liberated iodine was measured spectrophotometrically at 420 millimicrons. A blank reading was obtained by carrying out the procedure in the absence of agent. The spectrophotometer was adjusted to zero initially with distilled water.

Subtraction of the sample reading from the blank reading gave a value equivalent to the quantity of Agent V present.

3. Calibration Curve for Agent V

Analysis of a series of solutions containing known amounts of Agent V in aqueous 78 percent propylene glycol gave the calibration curve shown in Figure 6. It should be pointed out that this curve was obtained using an impure grade of Agent V (Levinstein, purity about 75 percent). It would have been desirable, perhaps, to have used a better grade of distilled agent, if available, but this poorer grade of material represented the product which was of interest militarily.

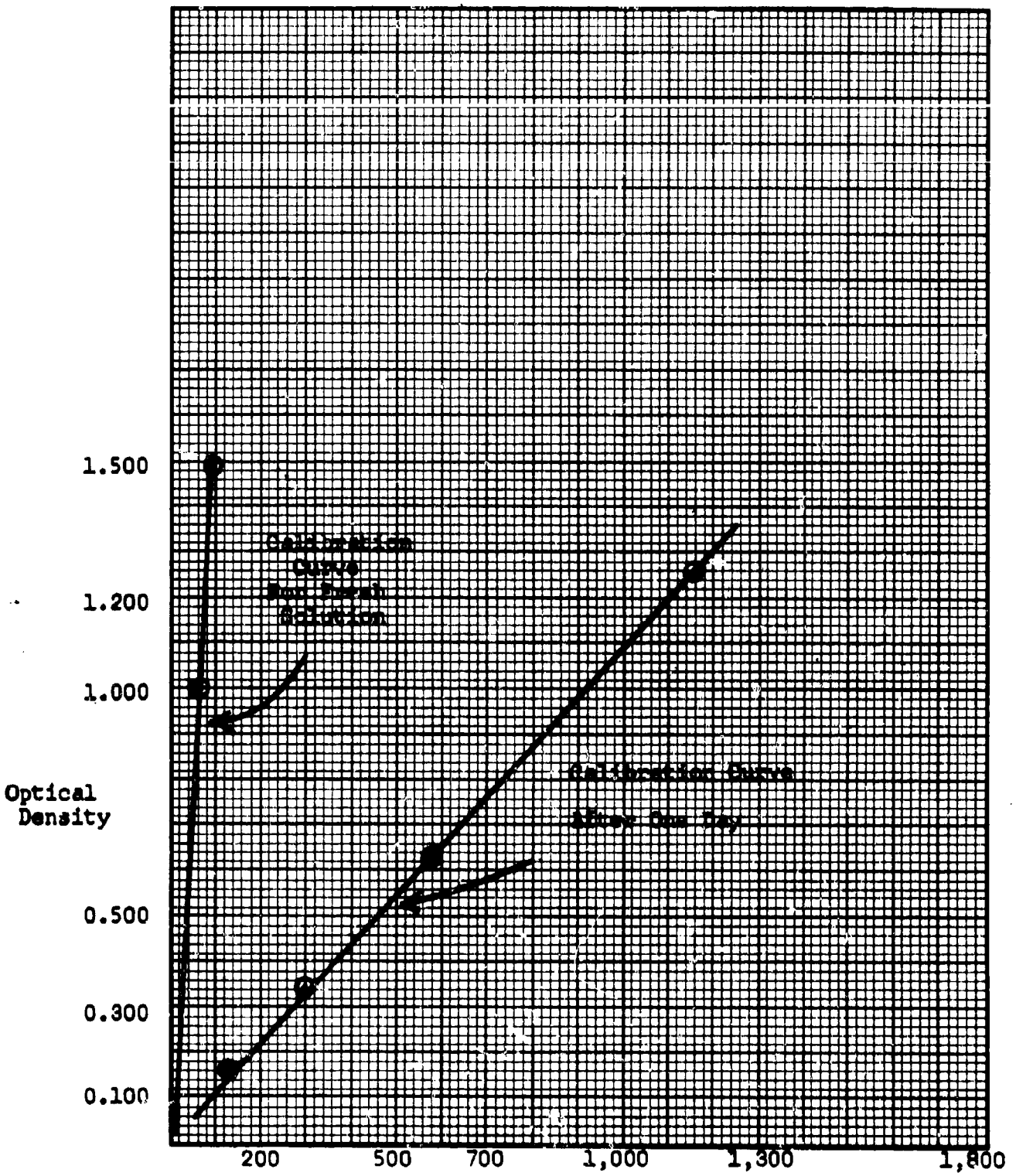


Figure 5 - Lack of Stability of Agent V in Aqueous Propylene Glycol.

V

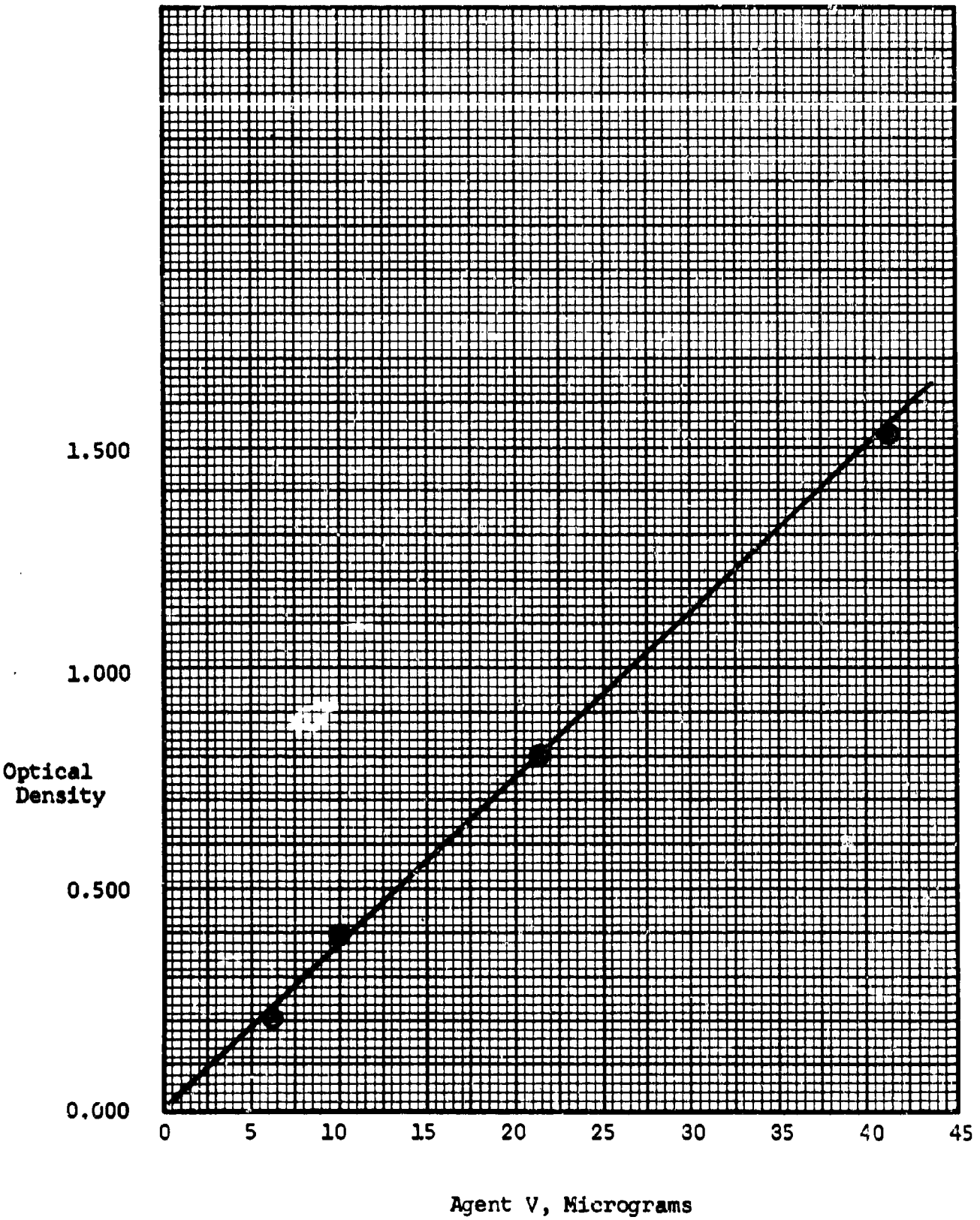


Figure 6 - Calibration Curve For Agent V
Using the Chloramine-T Procedure.

VI. EXPERIMENTAL PROCEDURES

A. Design and Construction of a Test Cell

The design of a suitable test cell by means of which the penetration of a test film by an agent could be measured was begun on the premise that the apparatus should consist of two adjacent chambers. The film to be tested would be inserted as a barrier between these two sections. The first chamber would be used for the generation of agent in the vapor or liquid phase. Measurement of the agent present in the second chamber at successive intervals would indicate the rate at which penetration of the intervening barrier had occurred.

The nature of the agents to be studied was found to impose restrictions on the design of the test cell. Certain agents have been found to decompose relatively completely in a period of several hours when a small quantity was allowed to contact a relatively extensive glass surface. It was necessary, therefore, to limit the over-all size of the test cell to be used and to restrict severely the contact of the agent vapor with glass walls. This was considered particularly important in the collection chamber, since it was planned to analyze the contents periodically over an indefinite period of perhaps several weeks.

In order to hold the agent over a period of time in the collection chamber and minimize any direct contact with the glass walls, the use of a collecting solution was proposed.

It was also considered necessary to limit the volume of solution in the collecting chamber since the amount of agent

penetrating the films could be expected to be probably quite small, and too large a volume of collecting solution might dilute the agent concentration beyond the limits of the analytical procedure.

Therefore, a shallow collecting dish was designed which would hold 20-25 ml. of collecting solution and which would leave only about one cm. or less path between the film and the surface of the liquid. A side arm was attached for removing liquid samples. A 3/8-inch magnetic stirring bar (Teflon coated) was placed in each dish for use in mixing the solutions before sampling.

The agent vapor generation chamber was designed as shown in Figure 1. A number of designs were tested in an attempt to secure a more uniform and perhaps higher concentration of agent vapor. This test cell, however, appeared to offer a simple, inexpensive system which would be readily adaptable to all of the types of films and packaging materials to be tested. The packaging materials could be inserted readily for testing and the atmosphere within the chamber could be analyzed intermittently as could the collecting solution.

In order to make the system as nearly isolated as possible, rubber septums were inserted in both sampling tubes. In order to avoid the creation of a vacuum within the system as samples were withdrawn, extra hypodermic needles were placed permanently in the edge of the rubber septums. As a sample was withdrawn, air was then allowed to enter through the extra needle as

FIGURE NO. 7 PERMEABILITY TEST CELL

required to maintain a constant pressure. After use of this system with the first test agent, it was decided that the use of rubber septums was unnecessary and small corks were used to seal the ends of the sampling tubes. The corks were removed during the sampling procedure. This led to more efficient cleaning and decontamination when the agent being used was changed.

It was desirable to carry out the tests at a relative humidity of about 50 percent. Since solutions of glycols in water can be used to maintain atmospheres at a desired relative humidity and propylene glycol, if properly diluted, was thought not to interfere seriously with the proposed enzymatic analytical procedure, a 78.5 percent solution of propylene glycol in water (B-4, B-5) was selected. Also present in the water when Agent I was used should be sufficient acid to maintain the collected agent as a salt, where possible, in order to minimize the partial pressure of the agent above the solution.

Some studies were also made of ethylene glycol for use in the collecting solution. There appeared to be less interference from ethylene glycol than from propylene glycol. Should interference from propylene glycol have proved to be extensive enough to affect the results obtained, ethylene glycol could have been used instead.

The collecting solution (78 percent aqueous propylene glycol) was analyzed daily, as far as possible, using the 1:10 serial dilution procedure in which 1.0 ml. was withdrawn, diluted to

10 ml. and analyzed. An aliquot of 1.0 ml. of this first dilution was again diluted to 10 ml. and analyzed. This 1:10 dilution process, as described in Section IV-D, page 49 was continued until an inhibition level below 100 percent was obtained.

The upper vapor chamber was analyzed similarly by removing one ml. of vapor with a syringe containing 1.0 ml. of 0.001 N sulfuric acid. After shaking the syringe thoroughly, the solution was diluted to 10.0 ml. with water and analyzed using the same 1:10 serial dilution procedure.

B. Procedure Used in Starting a Test Series

As shown in Figure 7, the upper or bell-shaped portion of the test cell was fitted with a rubber stopper at the top and a small cork at the opening in the side arm. This section was then suspended slightly above the position designated for the collecting dish section of the cell.

The collecting dish section was fitted with a small cork at the side arm opening and was then placed in position directly under the upper cell section. A small stirring bar was placed in the collecting dish and 35.0 ml. of a 78.5 percent solution of propylene glycol in water were added. Care was taken to ensure that none of this collecting solution was spilled on the ground glass surface at the edges of the dish.

A section of the film to be tested was then cut in a circular pattern,with a small protruding portion which was used for handling so as to eliminate the effects of finger marks or

perspiration. This section of film was cut so as to extend about one-quarter inch beyond the edge of the collecting dish to ensure complete coverage of the dish and to eliminate the effect of any stretching or tearing while cutting the film. The circular test section was then placed in position between the ground glass flanges of the collecting dish and the upper section. No attempt was made to pull the film tight nor was it allowed to sag.

1. Procedure for Starting Vapor Permeability Tests

If the series were to be a vapor permeation test, the upper portion of the cell was removed and a specially constructed 50 ml. beaker attached to a 6 mm. glass rod inserted as shown in Figure 7. This rod was used to suspend the beaker in the upper portion of the cell. Approximately 20.0 ml. of the agent to be tested were placed in the beaker. The upper cell section, with the rubber stopper removed, was then lowered over the beaker so that the glass rod extended approximately two inches through the opening at the top of the cell. A rubber stopper with a 7-8 mm. hole bored in the center was then placed snugly in the top opening of the cell section. This left approximately one inch of glass rod extending through the 7-8 mm. hole in the stopper. The glass rod was then grasped firmly and gently raised, so as to suspend the beaker about one and one-half inches above the level of the ground glass flange. While holding the glass rod at a point close to the rubber stopper, a one-inch piece of 5 mm.

rubber tubing was slipped over the glass rod so as to hold the beaker and agent at the desired height above the film. The smoothly cut edge of the tubing also forms a seal when in contact with the hold in the rubber stopper. The upper cell section containing the suspended beaker was then placed in position so that the ground glass surfaces of this section were just opposed to those of the collecting dish. After placing the film in position between the two ground glass surfaces, the flanges of the two cell sections were clamped together with paper clamps to ensure a tight seal. The whole assembly was then clamped to a rack in a suitable ventilating hood.

2. Procedure for Starting Liquid Permeability Test Series

When the series was to be a liquid permeability test, this same procedure was followed, except that the beaker assembly was not used. After the assembly was clamped in place, the stopper was removed and 0.04 ml. of the test agent was added through the opening in the top of the cell using a 0.05 ml. syringe and a six-inch curved needle. The needle was inserted into the cell through the opening and the contents of the syringe were expelled directly onto the film, keeping the needle tip approximately one inch above the film. The stopper was then replaced in order to seal the cell.

Care was taken to keep the cells level so as to prevent the agent from running to one side of the cell.

C. Procedure for Removal of Samples

1. Removal of Samples From the Collecting Solutions
The collecting solution to be analyzed was mixed thoroughly and carefully by moving a portable magnetic stirrer under the board supporting the test cell and spinning the 3/8-inch magnetic stirring bar which had been placed in the collecting solution at the start of the test. Care was taken that the mixing solution did not splash up to the underside of the material being tested.

The cork stopper was removed from the sampling tube and a suitable aliquot removed with a six inch blunted needle attached to a hypodermic syringe. The syringe was washed thoroughly before and after the sampling process in order to minimize contamination of subsequent samples. The washing stage was considered particularly important in view of the extreme differences observed in concentration of agent in the various collecting solutions. Disposable polyethylene syringes were used at times to eliminate contamination by the agents which were less easily washed out with water.

The sample of liquid removed was then diluted to 10 ml. as the first step in the dilution of the samples for analysis, as described in Section VI-D, page 50.

2. Removal of Samples From the Vapor Chamber
The cork in the side arm in the upper, or vapor, section of the test cell was removed and a 1.0 ml. sample of the atmosphere within the chamber was drawn into a hypodermic

syringe containing 1.0 ml. of a scrubbing solution. The syringe was then shaken vigorously and the contents were expelled beneath the surface of the solution used to make the first dilution used for the analysis.

The vapor sample was taken from an area within the chamber just above the outer edge of the beaker containing the agent supply. The scrubbing solution for the vapor samples was water except that, for samples of Agent I, 0.001 N H_2SO_4 was used.

D. Procedure for the Dilution of Samples

1. Prepare a series of dilutions of the solution to be tested. These dilutions may be prepared conveniently by dissolving the material to be tested in 10 ml. of water and diluting a 1.0 ml. aliquot of this solution to 10 ml. with water. A 1.0 ml. aliquot of this second solution is then diluted with water to 10 ml. This successive dilution process results in a series of solutions, each of which is one-tenth as concentrated as the preceding solution, and may be extended as far as necessary until a solution of the proper concentration is obtained. Usually, 5-10 successive dilutions are sufficient.

The 1:10 serial dilution procedure described in Section IV-B, above, for the preparation of calibration curves, has the disadvantage that only 1-2 points will be obtained which fall between about 20 and 80 percent inhibition. The number of calibration points obtained may be

increased, while maintaining the continuity of the 1:10 dilution procedure, by preparing one or two intermediate solutions involving a less than ten-fold dilution.

E. Enzymatic Procedure for the Determination of Anti-cholinesterase Activity

1. Principle

The enzymatic method of determining anticholinesterase activity is based upon the fact that acetylcholine is hydrolyzed by an enzyme, cholinesterase, which is present in mammalian blood. This hydrolysis of acetylcholine by the enzyme is a fundamental part of the mechanism by means of which impulses are transmitted through the nervous system. The toxicity of the nerve gas types of chemical warfare agent, for example, is based upon their ability to deactivate the enzyme and thus interrupt normal neuro-muscular processes.

The analytical procedure is based upon a measurement of the decrease in activity effected by the addition of such an anticholinesterase agent to a specified quantity of the enzyme. In actual practice, a reaction system is selected so that a controlled amount of enzyme will hydrolyze a measured quantity of acetylcholine. By adding solutions containing known amounts of cholinesterase inhibitor, the enzyme is partially inactivated and is correspondingly less effective in hydrolyzing the acetylcholine present. The acetylcholine remaining unhydrolyzed as a result of the inhibited activity of the enzyme is measured colorimetrically

by conversion to a colored ferric hydroxamate complex. A relationship can thus be developed between the amount of inhibitor known to be present in test solutions and the degree of inhibition of the activity of the enzyme. By comparison of the enzyme inhibiting strength of unknown solutions with the inhibiting effect of such known standard solutions, an estimation of the amount of inhibitor present in unknown solutions can be made.

2. Reagents

A. Buffer Solution (pH 7.2)	Dissolve 16.72 g. of $Na_2HPO_4 \cdot 12\ H_2O$ and 2.72 g. KH_2PO_4 in one liter of H_2O.
B. Acetylcholine	Dissolve about 0.1 g. of acetylcholine chloride in 100 ml. of buffer solution (A). Refrigerate.
C. Hydroxylamine Hydrochloride	Dissolve 13.9 g. $NH_2OH \cdot HCl$ in 100 ml. of H_2O; refrigerate.
D. NaOH Solution	14.0 g. NaOH dissolved in 100 ml. of distilled H_2O.
E. Alkaline Hydroxylamine	Mix one volume of reagent C with one volume of reagent D immediately before using.
F. HCl Solution	Dilute 100 ml. of concentrated aqueous HCl with 100 ml. of distilled H_2O.
G. 0.1 N HCl	Dilute 9.0 ml. of aqueous 37 percent HCl to 1 liter with distilled water.
H. Ferric Chloride Solution	Dissolve 10.0 g. $FeCl_3 \cdot 6\ H_2O$ in 100 ml. of solution G.
I. Cholinesterase Solution	Fresh human blood plasma or commercial Horse Serum (Fisher Scientific Co. 11/610) of the concentration indicated in Section 4-A below; refrigerate.
J. Trichloroacetic Acid Solution	Dissolve 15 g. of trichloroacetic acid, Cl_3CCOOH, in a mixture of 90 ml. of reagent F and 10 ml. of water.

3. Equipment

A. Syringes	Assorted syringes: 10 ml., 5.0 ml. and 1.0 ml., disposable polyethylene syringes, 2.0 ml. (Burron, Bethlehem, Pennsylvania).
B. Water Bath	Equipped with an agitator and thermostat so as to maintain a constant temperature of 37°C. ± 1°C.
C. Filter Paper	Whatman No. 1, or equivalent, 7.0 cm. diameter.
D. Test Tubes	Unstoppered, 15 x 150 mm. test tubes.
E. Test Tube Basket Support	Three, as required to suspend test tubes in the water bath and to hold test tubes during the dilution steps.
F. Spectrophotometer	B and L Spectronic 20, Fisher Cat. No. 7-144-1, or equivalent, with accessory test tubes, Fisher Cat. No. 7-144-15, as required.

4. Procedure

A. Standardization of Enzyme Solution

Since plasma cholinesterase varies in activity, it is necessary to adjust the concentration so as to prepare a solution of standardized enzyme activity. This concentration is chosen such that ca. 85 percent of the acetylcholine is hydrolyzed in one-half hour at 37°C. in the absence of inhibitor.

1. Dilute 2.5, 3.0, 3.5, 4.0 and 4.5 ml. of horse serum to 25 ml. with buffer solution (reagent A). These solutions may be labelled dilutions 1, 2, 3, 4 and 5.

2. Select seven 15 x 150 mm. test tubes and, for clarity, label these Control A, Control B, Control C-1, C-2, C-3, C-4 and C-5, successively. Add 3.0 ml. of distilled water to each of the seven test tubes. To Control A, also add 2.0 ml. of buffer solution (reagent A). To Control B, also add 1.0 ml. of buffer solution (reagent A).

3. Add 1.0 ml. of dilutions 1, 2, 3, 4 and 5 to Controls C-1, C-2, C-3, C-4 and C-5, respectively.

4. Place the tubes in the 37°C. constant temperature bath and allow to come to temperature equilibrium.

5. Add 1.0 ml. of acetylcholine solution (reagent B) to Control B and to Controls C-1, C-2, C-3, C-4 and C-5. Mix the contents of each tube and agitate mechanically while incubating for 30 minutes at 37°C.

6. Add 2.0 ml. of alkaline hydroxylamine solution (reagent E) to each of the tubes and mix thoroughly.

7. Add 1.0 ml. of trichloroacetic acid solution (reagent J) to each of the tubes and mix.

8. Filter the contents of each tube into graduates until at least 4.0 ml. of filtrate have been collected. Examine for clarity and, if turbid, refilter. Decant the excess filtrate over 4.0 ml.

9. Add 0.6 ml. of ferric chloride solution (reagent H) and mix. Measure the absorbance, as instructed below, of each solution before proceeding with the next solution.

10. Adjust the spectrophotometer to zero at 540 millimicrons with Control A. Measure the absorbance of each of the other solutions.

11. Calculate the percent hydrolysis as follows:

$$\frac{(D \text{ Control B} - D \text{ Control C})100}{D \text{ Control B}} = \% \text{ Hydrolysis}$$

Where D = absorbance (optical density)

12. Select a dilution of serum such that 1.0 ml. will effect hydrolysis of about 85 percent of the acetylcholine under these test conditions.

B. Determination of Cholinesterase Inhibition

1. Prepare a series of dilutions of the solution to be tested. These dilutions may be prepared conveniently by dissolving the material to be tested in 10 ml. of water and diluting a 1.0 ml. aliquot of this solution to 10 ml. with water. A 1.0 ml. aliquot of this second solution is then diluted with water to 10 ml. This successive dilution process results in a series of solutions, each of which is one-tenth as concentrated as the preceding solution, and may be extended as far as necessary until a solution of the proper concentration is obtained. Usually, 5-10 successive dilutions are sufficient.

2. Transfer a 3.0 ml. aliquot from each dilution to be tested to a 15 x 150 mm. test tube.

3. To each of three 15 x 150 mm. test tubes, labelled Control A, Control B and Control C, respectively, add 3.0 ml. of distilled water. To Control A, also add 2.0 ml. of buffer solution (reagent A). To Control B, also add 1.0 ml. of buffer solution (reagent A).

4. Place all the tubes, including at least Control C (Controls A and B need not be incubated except for operational convenience), in the constant temperature bath and add 1.0 ml. of serum solution (reagent I), to each of the sample tubes and to Control C (Not to A,B).

5. Incubate at 37°C. for 30 minutes.

6. To each of the sample tubes, and to Control B and Control C, add 1.0 ml. of acetylcholine solution (reagent B).

7. Incubate at 37°C. for 30 minutes.

8. To each of the tubes, including all three controls A, B and C, add 2.0 ml. of alkaline hydroxylamine solution (reagent E) and mix.

9. To each of the tubes, including all three controls A, B and C, add 1.0 ml. of trichloroacetic acid solution (reagent J) and mix.

10. Filter each solution, including the three controls, into graduates until at least 4.0 ml. of filtrate have been collected. Examine each filtrate for clarity and, if turbid, refilter. Decant the excess over 4.0 ml. from each filtrate.

(Note: In normal routine operation, Controls A and B need not be filtered. The excess over 4.0 ml. should be decanted from each.)

11. Add 0.6 ml. of ferric chloride solution (reagent H) and mix. Measure the absorbance immediately, as instructed below, before proceeding to the next sample.

12. Adjust the spectrophotometer to zero at 540 millimicrons with Control A. Measure the absorbance (optical density) of each of the other solutions at 540 millimicrons.

13. Calculate the percentage inhibition as follows:

$$\frac{(D \text{ unknown} - D \text{ Control C}) \, 100}{(D \text{ Control B} - D \text{ Control C})} = \text{Percent Inhibition}$$

Where D - absorbance (optical density)

14. By comparison with the calibration curve prepared using the inhibitor in question, determine the amount of inhibitor present in the 3.0 ml. aliquot used for the analysis. Apply the appropriate aliquot factors and calculate the concentration of inhibitor in the original sample. In general, an inhibition level between about 20 percent and about 80 percent should be obtained for best results.

C. Preparation of a Calibration Curve

1. A calibration curve is prepared by carrying out the procedure exactly as described in Section B, above, using a solution of known concentration for the preparation of the dilutions for testing.

2. Notes:

a. The calibration curve should be prepared, as far as possible, using a sample of inhibitor of the same quality and source as that being determined.

b. The calibration curve should be prepared, as far as possible, under the same conditions as the analytical determinations.

c. The 1:10 serial dilution procedure described in Section 4-B, above, for the preparation of dilutions for analysis, has the disadvantage that only 1-2 points will be obtained which fall between about 20 and 80 percent inhibition. The number of calibration points obtained may be increased, while maintaining the continuity of the 1:10 dilution procedure, by preparing one or two intermediate solutions involving a less than ten-fold dilution.

VII. EXPERIMENTAL RESULTS

The following tables describe these results.

Table XI

Permeation of Nineteen Packaging Materials by Agent I (Liquid) (a)
Replicate Determinations

Film No.	Material	Micrograms Permeated per 100 Sq. Inches							
		<10	$<10^2$	$<10^3$	$<10^4$	$<10^5$	$<10^6$	$<10^7$	Rating(b)
1	Sulphite Bd.					XX			5,5
2	Mylar		XX						2,2
3	Foil Laminate	XX							1,1
4	Kraft Bd.					XX			5,5
5	V3 Bd.		X	X					2,3
6	Saran 7	X	X						1,2
7	Saran 17	XX							1,1
8	Saran on Celloph.	XX	X						1,1,2
9	Saran Q4164.7				X	X			4,5
10	Cellophane				X	X			4,5
11	Foil Lam.-Dobeck-1	X							1
12	" " " -2	X							1
13	" " -MilPrint	X							1
14	" " -Con-Can	X							1
15	" " -Kleerpak	X							1
16	" " -MMM	X							1
17	Mylar (Dupont)			X					3
18	Saran on Celloph. (Am.Viscose)					X			5
19	Saran on Celloph. (Dupont)		X						2

(a) These results are expressed as the number of micrograms of agent passing through 100 sq.in. of film during the test. A constant quantity of about 750,000 micrograms ($<10^6$) per 100 sq.in. was used in each test.

(b) These ratings represent the logarithm (to the next highest unit value) of the amount of agent which would pass through 100 sq.in. of test film during the test.

Table XII

Permeation of Twenty-three Packaging Materials by Agent II (Liquid) (a)
Triplicate Determinations

Film No.	Material	Micrograms Permeated per 100 Sq. Inches							Rating (b) (c)
		<10	<10^2	<10^3	<10^4	<10^5	<10^6	<10^7	
1	Sulphite Bd.						X(X)	X	6,7,(6)
2	Mylar	(X)		X		X			3,5,(1)
3	Foil Laminate	(X)		XX					3,3,(1)
4	Kraft Bd.						X(X)	X	6,7,(6)
5	V3 Bd.		(X)	X	X				3,4,(2)
6	Saran 7		X	(X)		X			2,5,(3)
7	Saran 17			X(X)	X				3,4,(3)
8	Saran on Celloph.			(X)		XXX			5,5,(3),5
9	Saran Q4164.7				(X)	XX			5,5,(4)
10	Cellophane						XX(X)		6,6,(6)
11	Foil Lam.-Dobeck-1	XX							1,1
12	" " " -2	X							1
13	" " -MilPrint			X					3
14	" " -Con-Can	X					X		6,1
15	" " -Kleerpak	X							1
16	" " -MMM	X	X						1,2
17	Mylar (DuPont)	XX							1,1
18	Saran on Celloph. (Am.Viscose)					X	X		6,5
19	Saran on Celloph. (DuPont)			X	X				4,3
20	P.E. Low (DuPont)					X			5
21	P.E. Med (DuPont)				X				4
22	P.E. Low (U.C.)					X			5
23	P.E. Med (U.C.)				X				4

(a) These results are expressed as the number of micrograms of agent passing through 100 sq.in. of film during the test. A constant quantity of about 750,000 micrograms (<10^6) per 100 sq.in. was used in each test.

(b) These ratings represent the logarithm (to the next highest unit value) of the amount of agent which would pass through 100 sq.in. of test film during the test.

(c) Those numbers in parentheses represent the results of a third, and more reliable, series of tests. The improved results are particularly noticeable in those tests in which only small quantities of agent were apparently present (film Nos. 2 and 3).

Table XIII

Permeation of Twenty Packaging Materials by Agent III (Liquid) (a)
Duplicate Determinations

Film No.	Material	Micrograms Permeated per 100 Sq. Inches							Rating (b)
		<10	$<10^2$	$<10^3$	$<10^4$	$<10^5$	$<10^6$	$<10^7$	
1	Sulphite Bd.						X	X	6,7
2	Mylar	XX							1,1
3	Foil Laminate	XX							1,1
4	Kraft Bd.						XX		6,6
5	V3 Bd.		X	X					2,3
6	Saran 7	X		X					1,3
7	Saran 17	XX							1,1
8	Saran on Celloph.			X	X				3,4
9	Saran Q4164.7				X	X			4,5
10	Cellophane						XX		6,6
11	Foil Lam.-Dobeck-1			X					3
14	" " -Con-Can	X							1
16	" " -MMM	X							1
17	Mylar (DuPont)			X					3
18	Saran on Celloph. (Am.Viscose)					X			5
19	Saran on Celloph. (DuPont)				X				4
20	P.E. Low (DuPont)					X			5
21	P.E. Med (DuPont)					X			5
22	P.E. Low (U.C,)					X			5
23	P.E. Med (U.C.)					X			5

(a) These results are expressed as the number of micrograms of agent passing through 100 sq.in. of film during the test. A constant quantity of about 750,000 micrograms ($<10^6$) per 100 sq.in. was used in each test.

(b) These ratings represent the logarithm (to the next highest unit value) of the amount of agent which would pass through 100 sq.in. of test film during the test.

Table XIV

Permeation of Ten Packaging Materials by Agent IV (Liquid) (a)
Replicate Determinations

Film No.	Material	Micrograms Permeated per 100 Sq. Inches							
		<10	$<10^2$	$<10^3$	$<10^4$	$<10^5$	$<10^6$	$<10^7$	Rating(b)
1	Sulphite Bd.						XX		6,6
2	Mylar								(c)
3	Foil Laminate								(c)
4	Kraft Bd.						XX		6,6
5	V3 Bd.								(c)
6	Saran 7					X			5
7	Saran 17								(c)
8	Saran on Celloph.								(c)
9	Saran Q4164.7				XX				4,4
10	Cellophane					XX			5,5

(a) These results are expressed as the number of micrograms of agent passing through 100 sq.in. of film during the test. A constant quantity of about 750,000 micrograms ($<10^6$) per sq.in. was used in each test.

(b) These ratings represent the logarithm (to the next highest unit value) of the amount of agent, in micrograms, which would pass through 100 sq.in. of test film during the test.

(c) The analytical procedure used for the detection of Agent IV was not sufficiently sensitive to distinguish between film Nos. 2, 3, 5, 7 and 8.

Table XV

Permeation of Ten Packaging Materials by Agent V (Liquid) (a)
Duplicate Determinations

Film No.	Material	Micrograms Permeated per 100 Sq. Inches							
		<10	$<10^2$	$<10^3$	$<10^4$	$<10^5$	$<10^6$	$<10^7$	Rating(b)
1	Sulphite Bd.						XX		6,6
2	Mylar	X	X						1,2
3	Foil Laminate	X	X						1,2
4	Kraft Bd.						XX		6,6
5	V3 Bd.	X	X						1,2
6	Saran 7					XX			5,5
7	Saran 17				X	X			5,6
8	Saran on Celloph.				XX				5,5
9	Saran Q4164.7		X				X		2,6
10	Cellophane						XX		6,6

(a) These results are expressed as the number of micrograms of agent passing through 100 sq.in. of film during the test. A constant quantity of about 750,000 micrograms ($<10^6$) per 100 sq.in. was used in each test.

(b) These ratings represent the logarithm (to the next highest unit value) of the amount of agent, in micrograms, which would pass through 100 sq.in. of test film during the test.

Table XVI

Agent I

Vapor Permeability Test Series No. 1
Analyses of Vapor Chamber (a)

No. of Days	Material No. 1 Sulphite Bd.	2 Mylar	3 Alum. Lam.	4 Kraft	5 V3	6 Saran-7	7 Saran -17	8 Cello. Saran Ct'd.	9 Saran Q4164.7	10 Cello-phane
1			23	21	21	18	23	59	11	23
2	53	86	18	12	15	17	17	17	21	8
3	10	23	20	12	12	19	11	18	19	18
4			23	13	16	19	20	20	18	11
6	2	12								
7	2	10	10	3	7	5	7	7		11
8	4	4	5	2	2	8	5	7	15	6
9	8	6	2	1/2	1/2	2	3	1	7	2
13	1	4								
14	2	1								

(a) Results calculated as micrograms of Agent I per liter of atmosphere.

Table XVII

Agent I

Vapor Permeability Test Series No. 1
Analyses of Collecting Solutions (a, b)

	Material No.								
	1	2	3	4	5	6	7	8	10
No. of Days	Sul-phite Bd.	Mylar	Alum. Lam.	Kraft	V3	Saran-7	Saran -17	Cello. Saran Ct'd.	Cello-phane
1			(2.7)	3.3	(1.0)	(1.3)	(1.3)	(1.3)	800
2	2.8	(1.4)	(4.7)	4.7	(2.7)	(3.1)	(1.3)	(1.0)	5,100
3			(3.0)	6.5	(1.6)	(1.2)	(0.8)	(1.2)	3,900
4			(1.5)	8.2	(1.2)	(1.2)	(0.8)	(1.3)	560
5	138	(0.7)							
6	191/625	(1)							
7	1130	10	(1.3)	100	(1)	(1.3)	(0.6)	(1.3)	1,000
8	5400	7.3	(1.1)	98/320	(2.2)	(1.5)	(0.7)	(1.3)	1,100
9	1000/ 2100	(2.7)	(2.2)	100/ 300	(2.2)	(1.6)	(1.4)	(1.8)	910/ 1580
12	910	(4.0)							
13	1100	(2.5)							
14	1750	(2.2)							

(a) Results expressed as total micrograms of Agent I penetrating 100 sq.in. of material.

(b) The values less than about 3 micrograms shown are below the sensitivity level of the analytical procedure and are considered to be without significance. Values of doubtful significance are enclosed in parentheses.

Table XVIII

Agent I

Vapor Permeability Test Series No. 2
Analyses of Vapor Chamber (a)

	Material No.									
	1	2	3	4	5	6	7	8	9	10
No. of Days	Sul-phite Bd.	Mylar	Alum. Lam.	Kraft	V3	Saran-7	Saran -17	Cello. Saran Ct'd.	Saran Q4164.7	Cello-phane
1	7	15	11	5	6	7	2	15	19	5
2	10	17	17	20	11	8	10	23	43	21
5	3	3	3	3	17	2	10	10	4	3
6	6	4	5	(1)	8	4	7	6	3	2
7	5	3	5	3	(2)	9	4	3	(1/2)	4
9	(1)	(1)	(2)	(1/2)	(2)	3	3	(2)	(2)	5

(a) Results calculated as micrograms of Agent I per liter of atmosphere.

(b) The values shown in parentheses are considered to be without significance since they fall below the sensitivity level of the analytical procedure.

Table XIX

Agent I

Vapor Permeability Test Series No. 2
Analyses of Collecting Solution (a)

No. of Days	Material No. 1 Sul-phite Bd.	2 Mylar	3 Alum. Lam.	4 Kraft	5 V3	6 Saran-7	7 Saran -17	8 Cello. Saran Ct'd.	9 Saran Q4164.7	10 Cello-phane
1	(1.7)	(1.3)	(0.6)	2.7	(0.6)	(1.3)	(0.6)	(1.6)	(0.8)	2.0
2	3.1	6.6	(1.3)	(0.2)	(1.6)	(0.8)	(0.6)	(3.6)	(1.6)	14.2
5	102	(2.0)	(1.8)	65	(1.2)	(1.4)	(0.9)	(0.9)	(1.2)	650
6	124	(1.2)	(1.0)	90	(1.1)	(1.1)	(1.0)	(1.1)	(1.1)	965
7	120/ 310	(1.8)	(1.8)	85/ 200	(1.1)	(0.7)	(0.7)	(1.1)	(1.1)	760/ 1120
9	115/ 330	(1.1)	(1.1)	71/ 140	(1.1)	(1.1)	(1.1)	(0.7)	(1.6)	875/ 1000

(a) Results calculated as total micrograms of Agent No. 1 penetrating 100 sq.in. of test material. The values less than 3 micrograms are below the sensitivity limit of the analytical procedure and are considered to be without significance. Such values are enclosed in parentheses.

Table XX

Agent I

Liquid Permeability Test Series No. 1

Analyses of Collecting Solution

(Micrograms of Agent Permeated per 100 Square Inches) (a)

	Material No.									
	1	2	3	4	5	6	7	8	9	10
No. of Days	Sulphite Bd.	Mylar	Aluminum Laminate	Kraft	V3	Saran-7	Saran-17	Cellophane Saran Coated	Saran Q4164.7	Cellophane
1	1,070			1,180						
3	9,500			5,750					2,820	
6	7.700			6,500					6,000	3,350
7	8,750			9,010					6,800	5,000
8	16,900		5.5	1,126					8,850	6,210
10	9,650	(2.2)		7,720					8,560	6,740
13	12,000	22	3	11,600	21	(2)	(2)	(2)	11,500	8,800
14	26,000	92	4	9,390	106	(1.5)	4	4	11,000	10,600
15	25,800	94	5	11,150	85	(1.4)	5	5	10,100	9,900

(a) The results shown in parentheses are considered to be without significance, since they fall below the sensitivity of the analytical procedure.

Table XXI

Agent I

Liquid Permeability Test Series No. 2

Analyses of Collecting Solution

(Micrograms of Agent Permeated per 100 Square Inches) (a)

	Material No.									
	1	2	3	4	5	6	7	8	9	10
No. of Days	Sulphite Bd.	Mylar	Aluminum Laminate	Kraft	V3	Saran-7	Saran-17	Cellophane Saran Coated	Saran Q4164.7	Cellophane
1	1,150			638				0	5.3	
2	4,130	13		688	5.5	25.8	(0.6)	0		125
4	7,890			5,800		12.9		0	4,010	3,520
7		77	(2.4)	9,800				0	8,550	7,350
8	32,100	103	3.7	11,300	79	57	(2.2)	0	7,500	9,800
9	18,400	92	4.1	11,100	68	55	(2.2)	0	9,350	9,200

(a) The results shown in parentheses are considered to be without significance, since they fall below the sensitivity of the analytical procedure.

Table XXII

Agent I

Liquid Permeability Test Series No. 1

Analyses of Vapor Chamber

(Vapor Concentration in Micrograms per Liter) (a)

	Material No.									
No. of Days	1 Sulphite Bd.	2 Mylar	3 Aluminum Laminate	4 Kraft	5 V3	6 Saran-7	7 Saran-17	8 Cellophane Saran Coated	9 Saran Q4164.7	10 Cellophane
1	6	(1)	4	(1)	8	8	6	7	13	6
3	9	4	12	3	6	19	5	18	15	3
6	7	22	15	4	6	17	3	20	9	15
7	10	11	23	20	8	9	3	13	19	17
8	5	9	8	(3)	(1)	5	(2)	12	7	(1)
10	3	>23	8	14	(1)	6	6	18	5	4
13	(2)	0	8	5	0	4	(2)	8	(2)	3
14	17	14	(2)	6	0	0	(1)	9	3	3
16	4	15	3	3	(1)	(1)	(1)	20	(1)	3

(a) Values in parentheses are based on very low inhibitions and are of questionable significance.

Table XXIII

Agent I

Liquid Permeability Test Series No. 2

Analyses of Vapor Chamber

(Vapor Concentration in Micrograms per Liter) (a)

	Material No.									
	1	2	3	4	5	6	7	8	9	10
No. of Days	Sulphite Bd.	Mylar	Aluminum Laminate	Kraft	V3	Saran-7	Saran-17	Cellophane Saran Coated	Saran Q4164.7	Cellophane
1	11	23	22	20	16	14	22	22	21	7
2	10	23	20	15	11	23	22	19	13	4
4	7	23	20	5	8	20	20	20	20	5
7	9	0	0	6	(2)	(3)	3	(2)	8	7
8	(2)	(1)	0	4	(1)	(1)	0	21	8	4
10	(1)	0	0	(2)	(1)	(1)	0	12	8	(3)

(a) Values in parentheses are based on very low inhibitions and are of questionable significance.

Table XXIV

Agent II

Liquid Permeability Test Series No. 1

Analyses of Collecting Solutions (a)

	Material No.									
	1	2	3	4	5	6	7	8	9	10
No. of Days	Sulphite Bd.	Mylar	Aluminum Laminate	Kraft	V3	Saran-7	Saran-17	Cellophane Saran Coated	Saran Q4164.7	Cellophane
1	1,200,000	7,000	106	1,000,000	4,240	77	79	17,580	6,810	619,000
3	1,500,000	10,000	495	900,000	6,256	420	313	22,500	16,266	3,000,000
4	500,000,000	10,000	1,030	700,000	6,256	267	127	18,000	11,058	1,000,000
7	50,000,000	19,000	980	670,000	1,900	58	260	11,500	15,000	675,000
8	18,000,000	--	188	570,000	--	17	970	16,900	24,400	930,000
9	2,000,000	70	40	--	93	55	--	--	--	720,000
10	800,000	105	36	1,800,000	69	55	1,050	13,600	18,600	720,000

(a) Results expressed as total micrograms of Agent II in the collection solution based on 100 sq.in. of film.

For example, the magnitude of results listed are in error due to early calibration errors. Results are valid, however, when considered as comparative indications of barrier efficiencies.

Table XXV

Agent II

Liquid Permeability Test Series No. 2

Analyses of Collecting Solutions (a)

	Material No.									
	1	2	3	4	5	6	7	8	9	10
No. of Days	Sulphite Bd.	Mylar	Aluminum Laminate	Kraft	V3	Saran-7	Saran-17	Cellophane Saran Coated	Saran Q4164.7	Cellophane
2	2,000,000	6,250	681	722,000	2,061	51,600	500	25,000	28,800	619,000
3	1,400,000	31,300	626	1,000,000	7,250	105,000	1,060	20,000	31,300	1,300,000
4	700,000	20,000	200	800,000	4,000	58,000	900	20,000	31,300	500,000
7	580,000	--	200	380,000	1,900	58,000	920	27,000	52,000	580,000
8	5,600,000	4,563	200	1,300,000	9,402	56,000	500	33,650	60,500	940,000
10	690,000	--	160	1,200,000	--	--	700	24,500	53,000	720,000

(a) Results expressed as total micrograms of Agent II in the collecting solution based on 100 sq.in. of film.

For example, the magnitude of results listed are in error due to early calibration errors. Results are valid, however, when considered as comparative indications of barrier efficiencies.

Table XXVI

Agent II

Liquid Permeability Test Series No. 1

Analyses of Vapor Chamber (a)

	Material No.									
No. of Days	1 Sulphite Bd.	2 Mylar	3 Aluminum Laminate	4 Kraft	5 V3	6 Saran-7	7 Saran-17	8 Cellophane Saran Coated	9 Saran Q4164.7	10 Cellophane
1	35	2,300	7,600	35	8,900	>13,000	>13,000	1,700	7,300	35
3	>130	170	1,700	30	3,300	1,700	1,700	170	1,700	60
4	475	99	330	30	825	660	990	330	495	35
7	65	25	--	15	260	--	260	--	--	35
8	--	5	20	10	350	50	33	--	--	30
10	(2)	3	(1)	15	>130	50	>130	5	91	20

(a) Results expressed as micrograms of Agent II per liter of atmosphere in the vapor chamber.

Table XXVII

Agent II

Liquid Permeability Test Series No. 2

Analyses of Vapor Chamber (a)

	Material No.									
	1	2	3	4	5	6	7	8	9	10
No. of Days	Sulphite Bd.	Mylar	Aluminum Laminate	Kraft	V3	Saran-7	Saran-17	Cellophane Saran Coated	Saran Q4164.7	Cellophane
2	20	500	1,500	20	2,800	830	830	170	1,000	30
3	30	360	660	40	1,100	660	825	99	660	36
4	(2)	200	495	25	990	330	495	35	660	30
7	20	33	--	53	260	--	--	--	--	45
8	--	--	--	--	33	--	--	--	--	--
10	12	(1)	130	13	91	55	130	(3)	45	15

(a) Results expressed as micrograms of Agent II per liter of atmosphere in the vapor chamber.

Table XXVIII

Agent II

Liquid Permeability Test Series No. 3 (a)

Analyses of Collecting Solutions

	Material No.								
	1	2	3	4	5	6	7	8	9	10
No. of Days	Sulphite Bd.	Mylar	Aluminum Laminate	Kraft	V3	Saran-7	Saran-17	Cellophane Saran Coated	Saran Q4164.7	Cellophane
1	45,000	(1.0)[b]	(0.5)	42,000	(0.5)	(0.1)	(0.1)	13	100	420,000
2	62,000	6	(0.5)	400,000	4	(0.6)	(0.5)	200	40,000	510,000
6	140,000	5.5	(1.3)	40,000	5.5	(0.4)	20	80	1,400	600,000
10	100,000	4	(0.4)	480,000	13	110	400	180	3,800	680,000

(a) Results expressed as total micrograms permeated per 100 sq.in. of area.

(b) The values enclosed in parentheses represent low inhibition levels and are of questionable significance.

Table XXIX

Agent II

Vapor Permeability Test Series No. 1 (a)

Analyses of Collecting Solutions

No. of Days	Material No. 1 Sulphite Bd.	2 Mylar	3 Aluminum Laminate	4 Kraft	5 V3	6 Saran-7	7 Saran-17	8 Cellophane Saran Coated	9 Saran Q4164.7	10 Cellophane
1	210,000	65	(3) (b)	640,000	125	(4.5)	22	15	22,000	16,000
2	820,000	20	(0.8)	800,000	20	(0.2)	25	15,500	62,000	200,000
4	2,000,000	62	(2)	650,000	125	3,100	3,100	31,000	65,000	620,000
11	190,000	36	(4)	190,000	300	5,000	4,800	115,000	135,000	--

(a) Results expressed as total micrograms permeated per 100 sq. in. of area.

(b) The values enclosed in parentheses represent low inhibition levels and are of questionable significance.

Table XXX

Agent III

Liquid Permeability Test Series No. 1 (a)

Analyses of Collecting Solutions

	Material No.									
	1	2	3	4	5	6	7	8	9	10
No. of Days	Sulphite Bd.	Mylar	Aluminum Laminate	Kraft	V3	Saran-7	Saran-17	Cellophane Saran Coated	Saran Q4164.7	Cellophane
1	250,000	(3.5)[b]	(3.5)	100,000	(3.5)	(3.5)	(3.5)	(3.5)	(2)	140,000
2	310,000	(3.5)	(3.5)	200,000	18	(3.5)	(3.5)	(3.5)	(3.5)	250,000
5	300,000	(9)	(4)	250,000	13	(4)	(5)	140	600	250,000
6	420,000	(9)	(5)	200,000	30	(5)	(4)	120	1,000	240,000
7	300,000	(9)	(5)	250,000	9	(7)	(4)	110	1,800	--
9	--	(3)	(3)	--	(3)	(3)	(3)	110	2,000	--

(a) Results expressed as total micrograms permeated per 100 sq.in. of area.

(b) The values enclosed in parentheses represent low inhibition levels and are of questionable significance.

Table XXXI

Agent III

Liquid Permeability Test Series No. 2 (a)

Analyses of Collecting Solutions

No. of Days	Material No. 1 Sulphite Bd.	2 Mylar	3 Aluminum Laminate	4 Kraft	5 V3	6 Saran-7	7 Saran-17	8 Cellophane Saran Coated	9 Saran Q4164.7	10 Cellophane
1	820,000	(2)[b]	(2)	80,000	(3)	(5)	(3.5)	320	50	210,000
2	2,600,000	(5)	(5)	270,000	(5.5)	(5.5)	(3.5)	2,000	--	--
6	600,000	(7)	(3.5)	240,000	60	60	(4)	1,850	2,700	360,000
7	1,300,000	(5)	(4)	360,000	55	55	(4)	1,000	2,500	250,000
9	--	(5)	(5)	--	110	110	--	930	11,000	--

(a) Results expressed as total micrograms permeated per 100 sq.in. of area.

(b) The values enclosed in parentheses represent low inhibition levels and are of questionable significance.

Table XXXII

Agent IV

Liquid Permeability Test Series No. 1 (a)

Analyses of Collecting Solutions

	Material No.									
	1	2	3	4	5	6	7	8	9	10
No. of Days	Sulphite Bd.	Mylar	Aluminum Laminate	Kraft	V3	Saran-7	Saran-17	Cellophane Saran Coated	Saran Q4164.7	Cellophane
1	54,000	--	--	7,600	--	--	--	--	--	2,500
2	115,000	(65) (b)	(25)	25,000	(65)	5,500	(65)	(65)	(65)	18,000
3	190,000	--	--	89,000	--	6,700	--	--	--	22,000
4	250,000	(125)	--	104,000	--	14,700	--	--	550	43,000
7	470,000	--	--	200,000	--	20,000	--	--	2,250	80,000
8	482,000	--	--	210,000	--	23,000	--	--	--	83,000

(a) Results expressed as total micrograms permeated per 100 sq.in. of area.

(b) The values enclosed in parentheses represent low inhibition levels and are of questionable significance.

Table XXXIII

Agent IV

Liquid Permeability Test Series No. 2 (a)

Analyses of Collecting Solutions

	Material No.									
	1	2	3	4	5	6	7	8	9	10
No. of Days	Sulphite Bd.	Mylar	Aluminum Laminate	Kraft	V3	Saran-7	Saran-17	Cellophane Saran Coated	Saran Q4164.7	Cellophane
1	76,000	--	--	30,000	--	--	--	--	--	2,500
2	170,000	--	--	75,000	--	--	--	--	--	12,500
3	260,000	--	--	96,000	--	--	--	--	--	20,000
4	350,000	(125)(b)	(65)	134,000	--	--	(145)	--	250	31,000
7	480,000	--	--	210,000	--	--	--	--	1,500	52,000
8	650,000	--	--	233,000	--	--	--	--	1,900	80,000

(a) Results expressed as total micrograms permeated per 100 sq.in. of area.

(b) The values enclosed in parentheses represent low inhibition levels and are of questionable significance.

Table XXXIV

Agent V

Liquid Permeability Test Series (a)

Analyses of Collecting Solutions

	Material No.									
	1	2	3	4	5	6	7	8	9	10
No. of Days	Sulphite Bd.	Mylar	Aluminum Laminate	Kraft	V3	Saran-7	Saran-17	Cellophane Saran Coated	Saran Q4164.7	Cellophane
2	260,000	--	--	380,000	--	--	--	--	--	310,000
5	250,000	0	0	500,000	0	13,000	18,000	18,000	17,000	470,000
6	315,000	0	0	600,000	--	--	--	--	--	350,000
7	260,000	0	0	380,000	0	27,000	27,000	25,000	250,000	290,000
8	350,000	0	0	360,000	0	27,000	145,000	53,000	330,000	350,000
9	435,000	--	--	400,000	--	76,000	90,000	31,000	325,000	400,000

(a) Results expressed as total micrograms permeated per 100 sq.in. of area.

Table XXXV

Agent V

Liquid Permeability Test Series (a)

Analyses of Collecting Solutions

	Material No.									
	1	2	3	4	5	6	7	8	9	10
No. of Days	Sulphite Bd.	Mylar	Aluminum Laminate	Kraft	V3	Saran-7	Saran-17	Cellophane Saran Coated	Saran Q4164.7	Cellophane
2	180,000	--	--	420,000	--	--	--	--	--	0
5	310,000	0	0	450,000	0	19,000	19,000	13,000	--	120,000
6	400,000	0	0	550,000	0	--	--	--	--	305,000
7	340,000	0	0	430,000	0	24,000	24,000	12,000	0	360,000
8	350,000	0	0	280,000	0	33,000	33,000	27,000	0	270,000
9	400,000	--	--	400,000	--	63,000	87,000	24,000	--	360,000

(a) Results expressed as total micrograms permeated per 100 sq. in. of area.

Table XXXVI

Agent I

Liquid Permeability Test Series (a)(b)

Analyses of Collecting Solutions

No. of Days	Material No. 11 Aluminum Laminate Dobeck.1	12 Aluminum Laminate Dobeck.2	13 Aluminum Laminate MilPrint	14 Aluminum Laminate Con.Can	15 Aluminum Laminate Kleerpak	16 Aluminum Laminate MMM	17 Mylar	18 Cellophane Saran Ct'd. Am.Vis.	19 Cellophane Saran Ct'd. du Pont	8 Cellophane Saran Coated
1	(0.2)	(0.2)	(0.2)	(0.2)	(0.2)	(0.2)	(0.2)	(0.5)	2	(0.6)
4	(0.2)	(0.2)	(0.2)	(0.2)	(0.2)	(0.2)	36	--	27	2.2
5	--	--	--	--	--	--	330	--	29	2.7 12.0
6	--	--	--	--	--	--	--	1,000	--	--
7	(0.6)	(0.3)	(0.2)	(0.3)	(0.4)	(0.2)	200	1,200	20	22

(a) Results expressed as total micrograms permeated per 100 sq.in. of area.

(b) The values enclosed in parentheses represent low inhibition levels and are of questionable significance.

Table XXXVII

Agent II

Liquid Permeability Test Series (a)(b)

Analyses of Collecting Solutions

No. of Days	Material No. 11 Aluminum Laminate Dobeck.1	12 Aluminum Laminate Dobeck.2	13 Aluminum Laminate MilPrint	14 Aluminum Laminate Con.Can.	15 Aluminum Laminate Kleerpak	16 Aluminum Laminate MMM	17 Mylar	18 Cellophane Saran Ct'd. Am.Vis.	19 Cellophane Saran Ct'd. du Pont	8 Cellophane Saran Coated
1	(1)	(1)	--	--	--	--	--	--	20	6
4	(1)	(1)	(6)	62	(1)	(1)	(1)	14,500	60	60
5	(1)	(1)	(13)	600	(6)	(1)	(1)	110,000	420	420
6	--	--	--	18,000	(2)	--	--	--	--	--
7	(1)	(1)	380	>660,000	(16)	(1)	(1)	>660,000	1,350	75,000

(a) Results expressed as total micrograms permeated per 100 sq.in. of area.

(b) The values enclosed in parentheses represent low inhibition levels and are of questionable significance.

Table XXXVIII

Agent III

Liquid Permeability Test Series (a)(b)

Analyses of Collecting Solutions

No. of Days	Material No. 11 Aluminum Laminate Dobeck.1	14 Aluminum Laminate Con. Can	16 Aluminum Laminate MMM	17 Mylar	18 Cellophane Saran Ct'd. Am.Vis.	19 Cellophane Saran Ct'd. du Pont	20 P.E. Low du Pont	21 P. E. Med. du Pont	22 P.E. Low U.C.	23 P. E. Med. U.C.
2	(0.1)	(0.1)	(0.1)	(0.2)	2,900	12	3,200	3,200	10,000	1,650
6	(0.1)	(0.1)	--	8.2	14,200	125	31,000	4,100	31,000	4,100
7	(0.1)	(0.1)	~80	1.2	10,000	160	31,000	7,000	10,000	7,000
8	--	--	29	--	--	--	--	--	--	--

(a) Results expressed as total micrograms permeated per 100 sq.in. of area.

(b) The values enclosed in parentheses represent low inhibition levels and are of questionable significance.

Table XXXIX

Agent III

Liquid Permeability Test Series (a)(b)

Analyses of Collecting Solutions

No. of Days	Material No. 11 Aluminum Laminate Dobeck.1	14 Aluminum Laminate Con. Can	16 Aluminum Laminate MMM	17 Mylar	18 Cellophane Saran Ct'd. Am. Vis.	19 Cellophane Saran Ct'd. du Pont	20 P. E. Low du-Pont	21 P. E. Med. du Pont	22 P. E. Low U. C.	23 P. E. Med. U. C.
2	100 320	(3)	(3)	65	32,000	(0)	7,300	3,500	10,000	4,200
6	--	5	(4)	310	31,000	--	31,000	10,000	31,000	12,300
7	110	5	5	--	50,000	1,800	78,000	30,000	78,000	30,000
8	--	--	--	380	--	--	--	--	--	--

(a) Results expressed as total micrograms permeated per 100 sq.in. of area.

(b) The values enclosed in parentheses represent low inhibition levels and are of questionable significance.

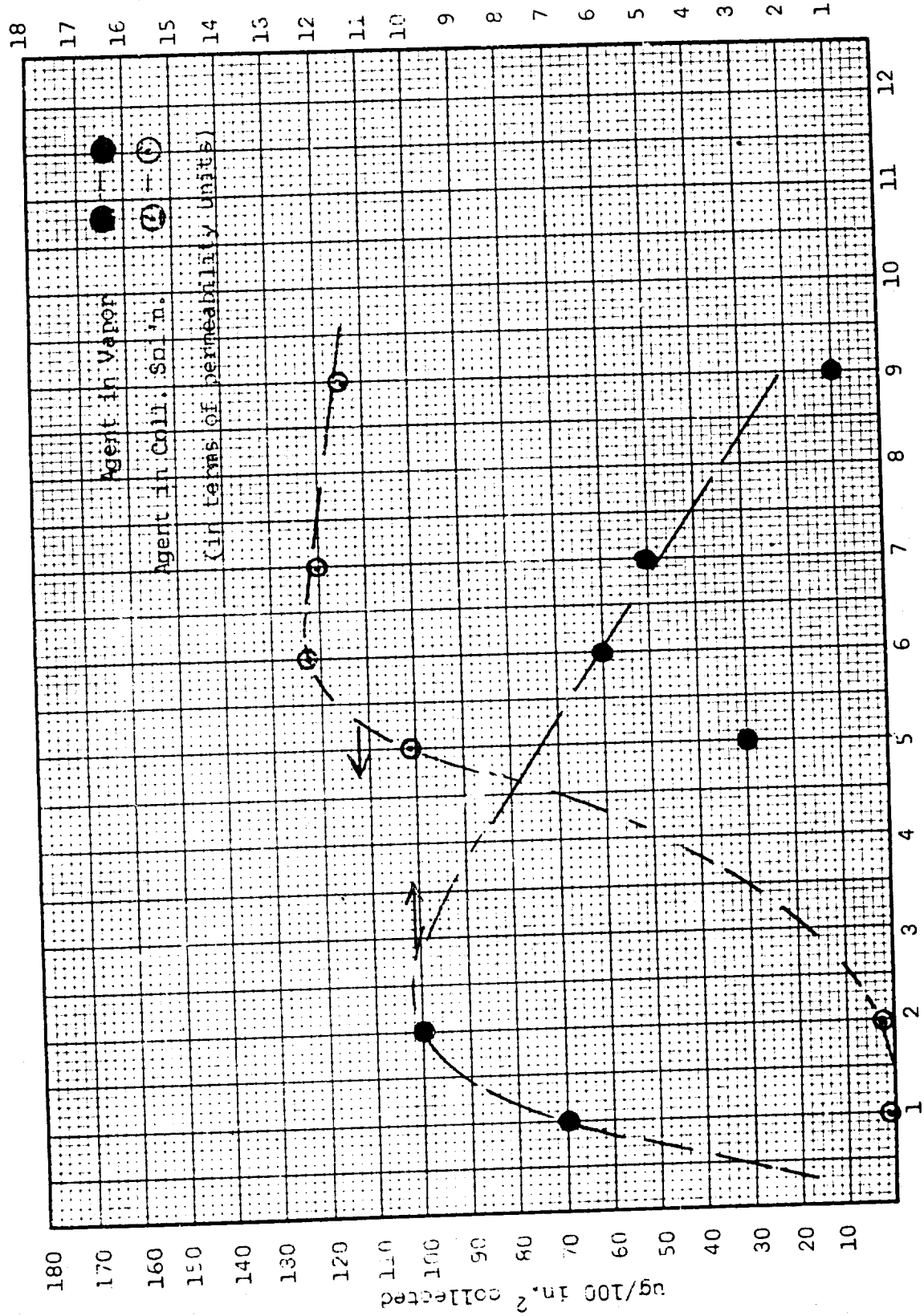


Figure 8 - Test Material No. 1, Series No. 2, Agent I Vapor Tests
White Sulphite Board

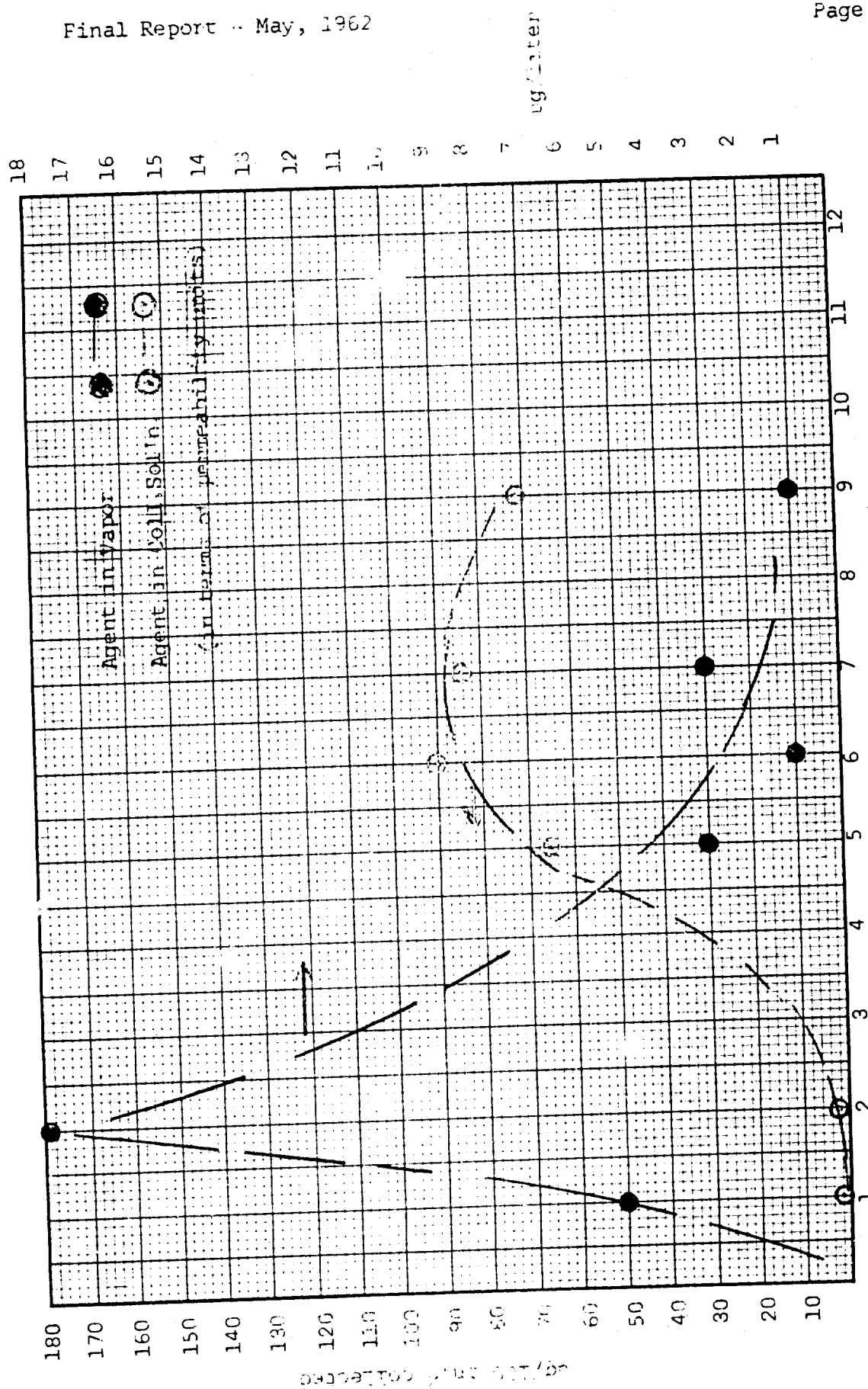
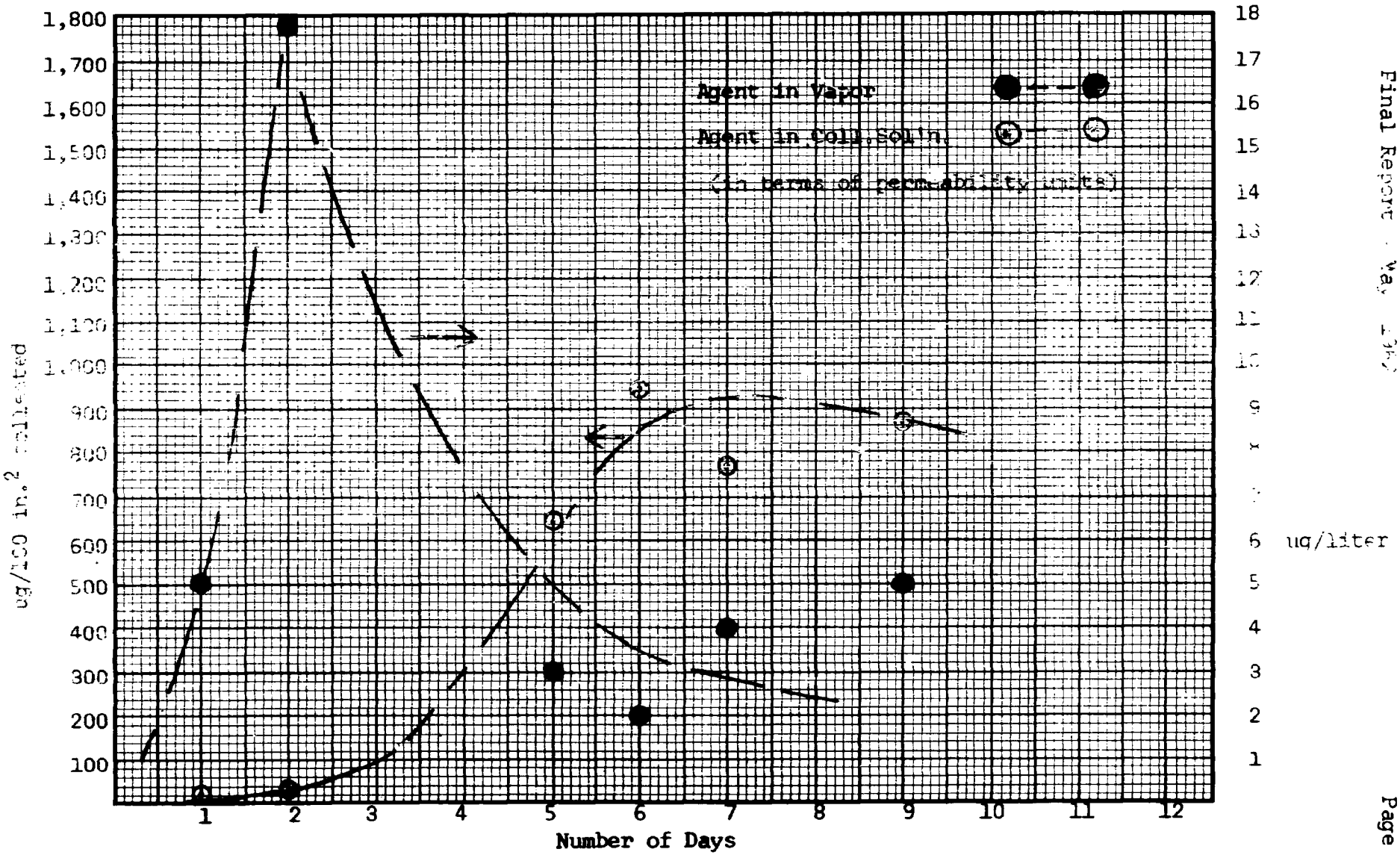


Figure 9 - Test Material No. 4, Series No. 2, Agent I Vapor Tests

Kraft Board



ug/200 in.^{2} collected

Figure 10 - Test Material No. 10, Series No. 2, Agent I Vapor Tests

Cellophane

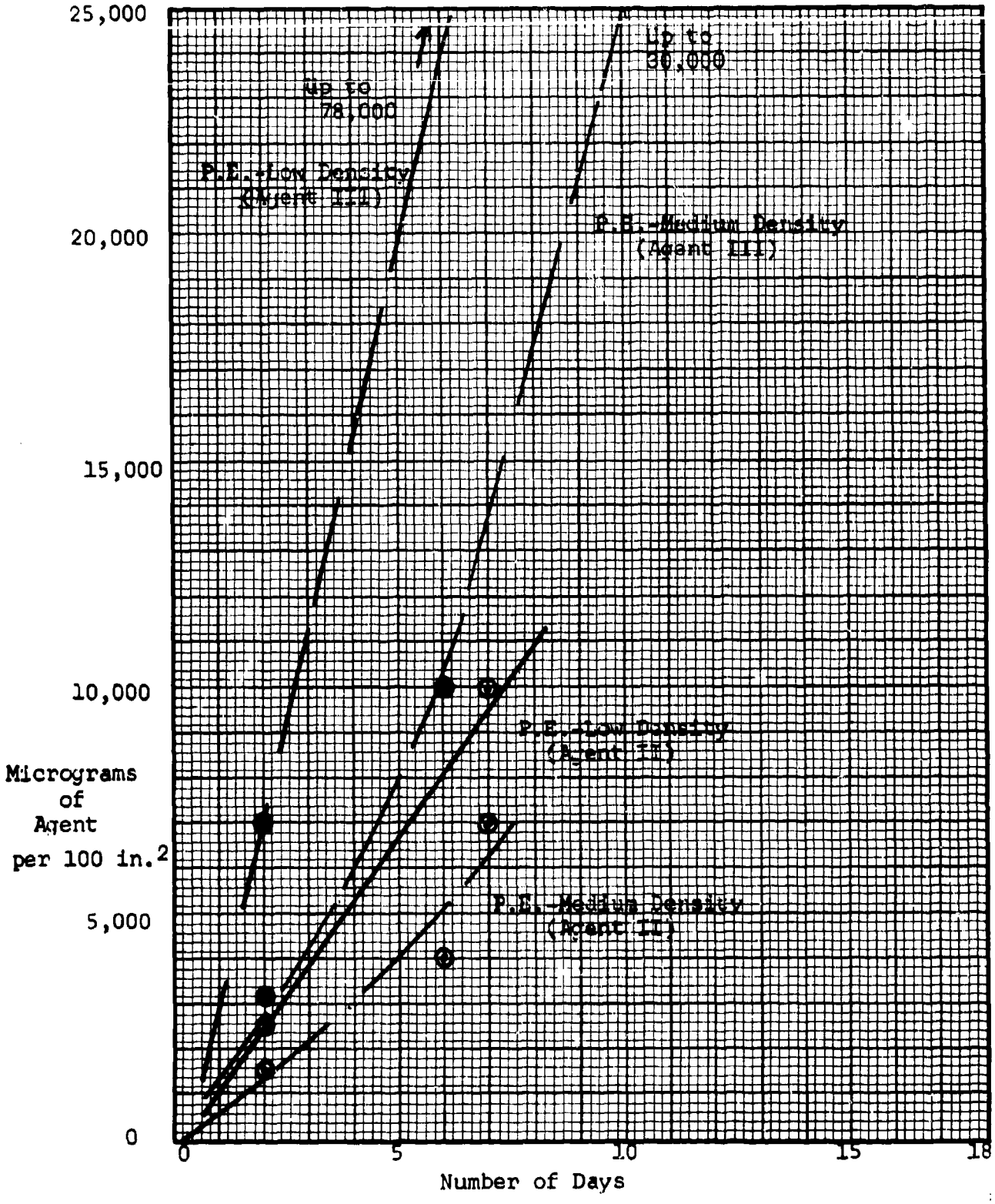


Figure 11 - Liquid Permeability of Materials 20, 21, 22 and 23 Toward Agents II and III.

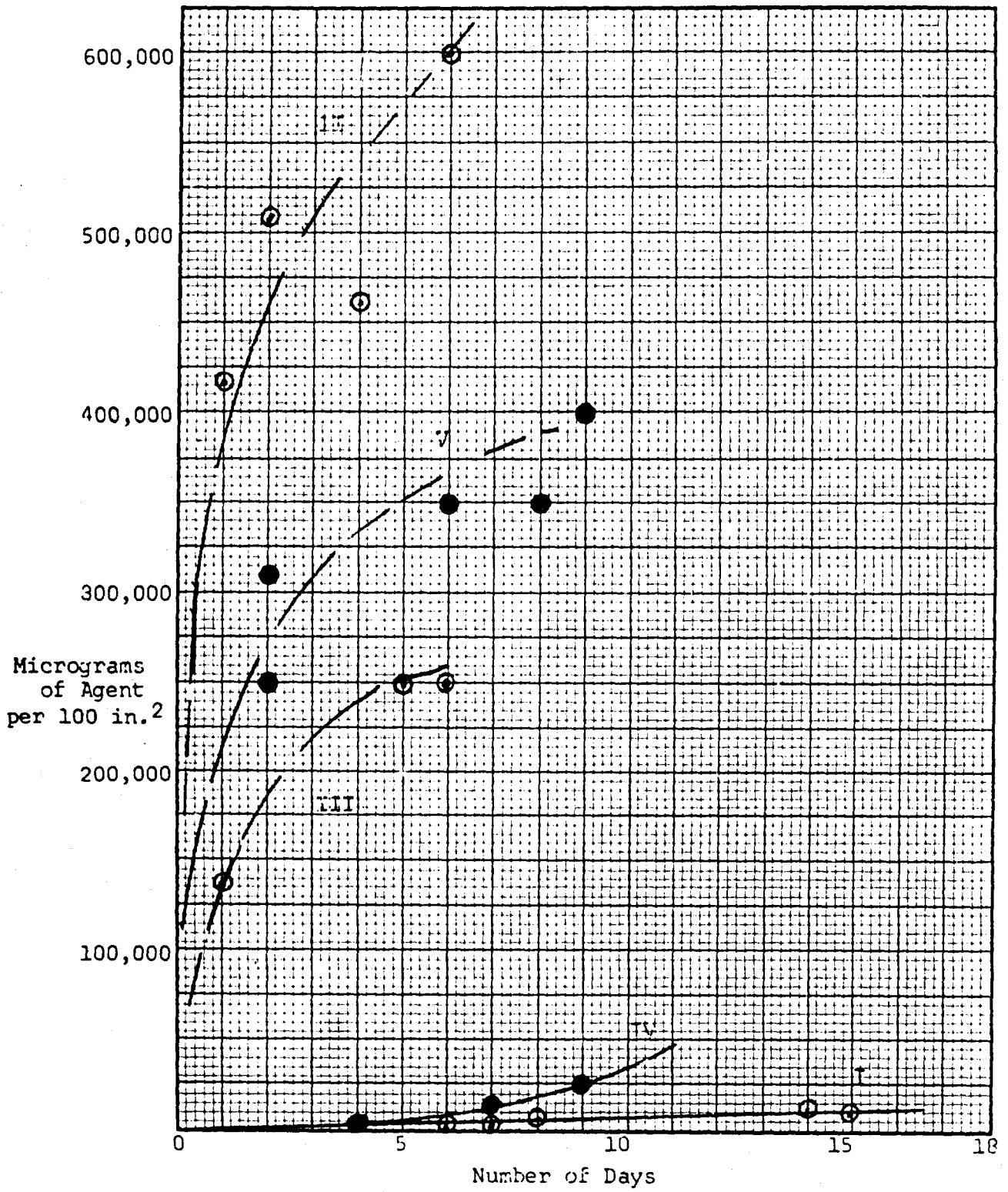


Figure 12 - Liquid Permeability of Test Material 10 (cellophane) To Agents I through V.

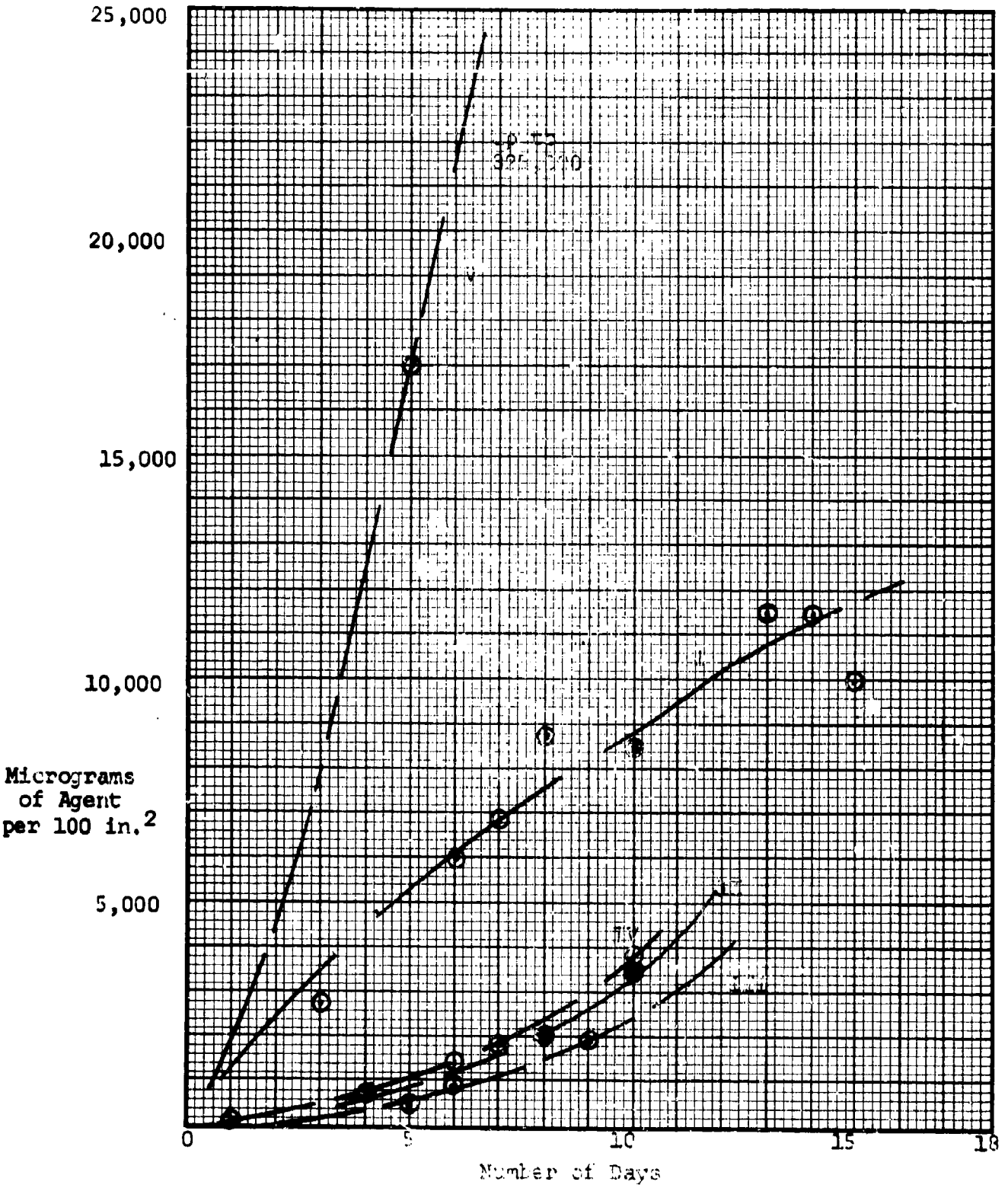


Figure 13 - Liquid Permeability of Test Material 9, Saran, Q4164.7, Toward Agents I through V.

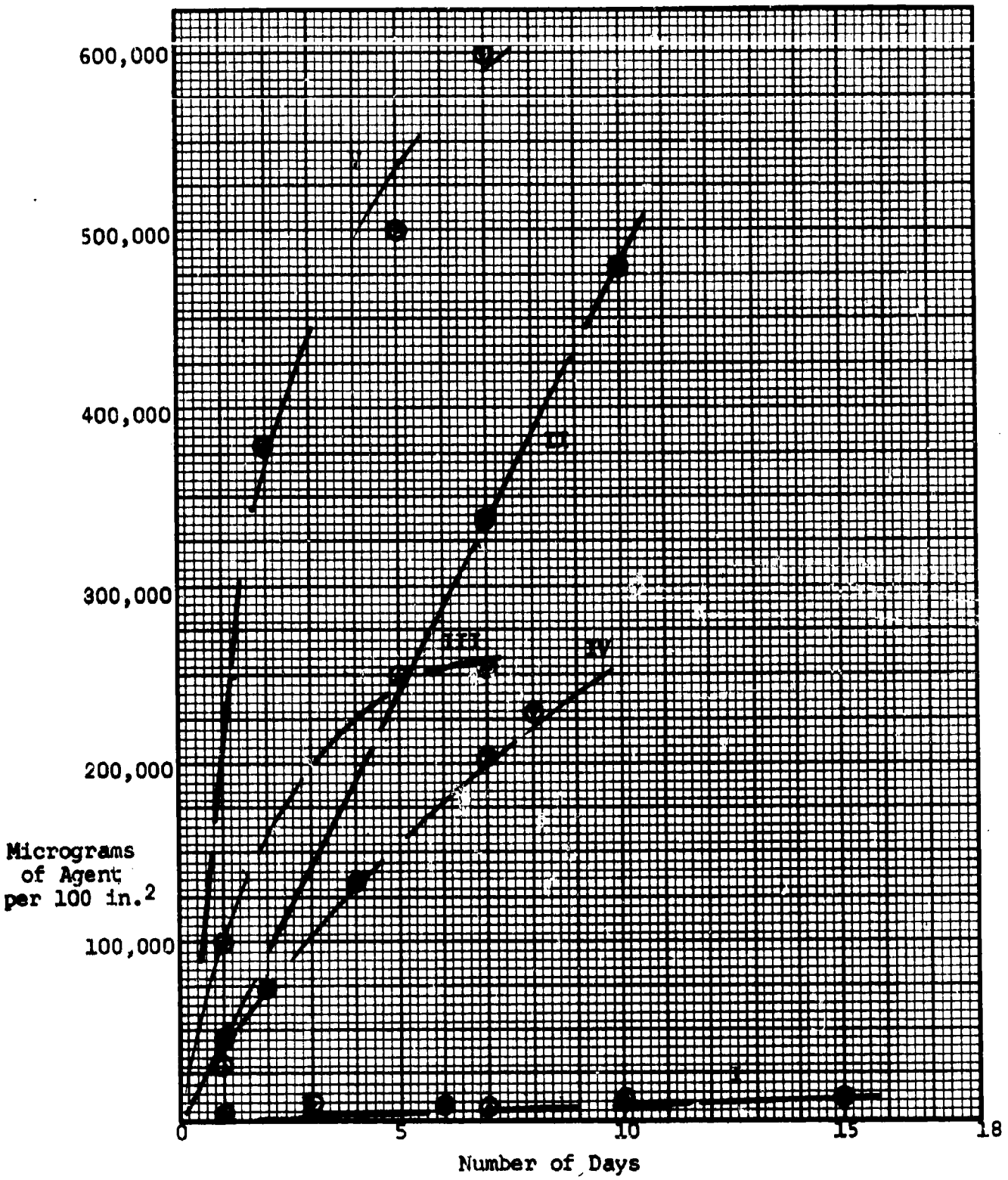


Figure 14 - Liquid Permeability of Test Material 4, Kraft board, Toward Agents I through V.

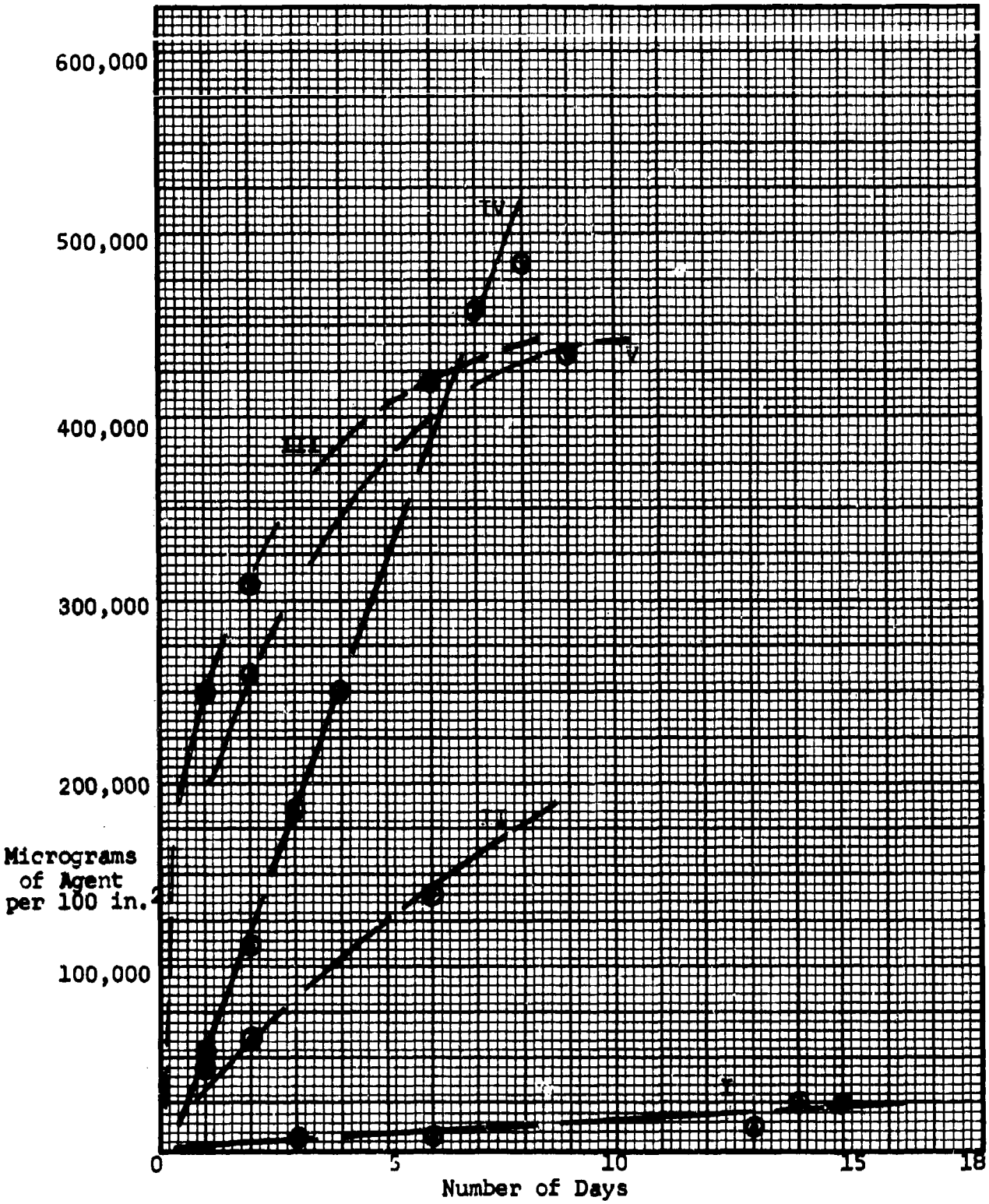


Figure 15 - Liquid Permeability of Test Material 1, White Sulphite board, Toward Agents 1 through V.

VIII. LITERATURE CITED

A. FMC Literature

1. Research Notebook No. D-495, pages 3 through 150, inclusive.

2. Research Notebook No. D-590, pages 2 through 150, inclusive.

3. Research Notebook No. D-616, pages 3 through 93, inclusive.

B. Published Literature

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4. Dow Glycols, 1947, Dow Chemical Company, Midland, Michigan.

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AD ______ Accession No. ______

FMC Corporation, Baltimore, Maryland
A STUDY OF THE RESISTANCE OF CONTAINERS TO CHEMICAL WARFARE AGENTS - John I. Stevens, Edward F. Orwoll

Report No. Final May, 1962, 95 pp. - 15 illus., 39 tables.
(Contract No. DA19-129-QM-1784)
Project No. 7-91-03-015, OI No. 5091,

Twenty-three samples representing nine different types of packaging materials were tested for resistance to permeation by five selected chemical warfare agents. A test system was developed which permitted a specific agent to be placed directly in contact with the test film. The permeability of the film was then estimated by collecting and measuring the agent which was found to pass through the film into an adjacent chamber.

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2. Packaging Films
3. Chemical Warfare Agents
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